DOCUMENT RESUME

ED 153 866 SE 024 250

TITLE	Basic Laboratory Skills. Training Module 5.300.2.77.
INSTITUTION	Kirkwood Community Coll., Cedar Rapids, Iowa.
SPONS AGENCY	Department of Labor, Washington, D.C.; Iowa State Dept. of Environmental Quality, Des Moines.
PUB DATE	Sep 77
NOTE	195p.; For related documents, see SE 024 249-254
EDRS PRICE	MF-$0.83 HC-$10.03 Plus Postage.
DESCRIPTORS	*Biology; *Chemistry; *Instructional Materials; *Laboratory Procedures; *Laboratory Techniques; Post Secondary Education; Secondary Education; Units of Study; Water Pollution Control; Water Resources
IDENTIFIERS	*Waste Water Treatment; *Water Treatment

ABSTRACT

This document is an instructional module package prepared in objective form for use by an instructor familiar with the basic chemical and microbiological laboratory equipment and procedures used in water and wastewater treatment plant laboratories. Included are objectives, instructor guides, student handouts and transparency masters. This module considers lab safety, bench sheets, labeling, sampling, solutions, dilution techniques, incubators, balances, glassware, standardization, standard curves, equipment packaging, autoclaves, microscopes and aseptic techniques. (Author/RH)

****
* Reproductions supplied by EDRS are the best that can be made *
* from the original document. *
****

ED153866

U.S. DEPARTMENT OF HEALTH,
EDUCATION & WELFARE
NATIONAL INSTITUTE OF
EDUCATION

THIS DOCUMENT HAS BEEN REPRODUCED EXACTLY AS RECEIVED FROM THE PERSON OR ORGANIZATION ORIGINATING IT. POINTS OF VIEW OR OPINIONS STATED DO NOT NECESSARILY REPRESENT OFFICIAL NATIONAL INSTITUTE OF EDUCATION POSITION OR POLICY

BASIC LABORATORY SKILLS

Training Module 5.300.2.77

"PERMISSION TO REPRODUCE THIS MATERIAL HAS BEEN GRANTED BY

Mary Jo Bruett

TO THE EDUCATIONAL RESOURCES INFORMATION CENTER (ERIC) AND USERS OF THE ERIC SYSTEM."

Prepared for the

Iowa Department of Environmental Quality
Wallace State Office Building
Des Moines, Iowa 50319

by

Kirkwood Community College
6301 Kirkwood Boulevard, S. W.
P. O. Box 2068
Cedar Rapids, Iowa 52406

The publication of these training materials was financially aided through a contract between the Iowa Department of Environmental Quality and the Office of Planning and Programming, using funds available under the Comprehensive Employment and Training Act of 1973. However, the opinions expressed herein do not necessarily reflect the position or policy of the U. S. Department of Labor, and no official endorsement by the U. S. Department of Labor should be inferred.

SE 024 250

September, 1977

The mention of trade names, or use of manufacturers technical bulletins, diagrams depicting specific equipment, or the commercial product in this module is for illustration purposes, and does not constitute endorsement or recommendation for use by Kirkwood Community College nor by the Iowa Department of Environmental Quality.

SUMMARY Page 1 of 168

Module No:

Module Title:

Basic Laboratory Skills

Submodule Titles

1. General Skills
2. Chemistry Skills
3. Microbiology Skills

Approx. Time:

33 hours

Objectives:

Upon completion of this module, the participant should be able to:

Perform and understand basic laboratory skills necessary as background information for microbiological or chemical analysis of water and wastewater samples.

Instructional Aids:

Handouts
Transparancies
Equipment used in laboratory practice

Instructional Approach:

Lecture
Discussion
Demonstration
Laboratory practice

References:

See individual topics

Class Assignments:

Read handouts
Participate in laboratory practice sessions

Module No:

Topic:
SUMMARY

Instructor Notes:

Basic Laboratory Skills

General Skills

- Safety
- Notebooks & Bench Sheets
- Labeling
- Sampling
- ID of lab equipment & glassware
- Chemical names and formulas
- Matter (solids)
- Solutions
- Dilution techniques
- Incubators
- Balances

Chemistry Skills

- Analytical analysis
- Volumetric glassware
- Standardization of reagents
- Colorimetric analysis
- Standard curves
- Lab supplies & chemicals
- Standard References

Microbiology Skills

- Laboratory cleanliness
- Equipment packaging
- Media & reagent preparation
- Sterilization
- Microscopes
- Aseptic technique
- Microbiological sample collection
- Microbiological dilution techniques

Instructor Outline:

1. Discuss, demonstrate and have student participate in laboratory practice and sessions concerning basic laboratory skills

Instructor must provide all necessary equipment for laboratory practice sessions. Necessary information or what equipment needed in handout materials.

Module No:

Module Title:
Basic Laboratory Skills

Submodule Title:
General Skills

Approx. Time:
1 hour

Topic:
Safety

Objectives:

Upon completion of this module, the participant should be able to:

1. Locate the following in the laboratory and indicate its proper use: Safety shower, fire extinguisher, fire blanket, eye wash, first aid kit and instruction sheet, fume hood.
2. Select the proper pieces of equipment given an emergency situation.
3. State when safety glasses, lab aprons and lab gloves will be used.
4. Given a list of common lab chemicals state their safety hazard and proper storage method.

Instructional Aids:

Handout: Laboratory Safety
Fire extinguishers A, BC, ABC, OCD
Fire blanket
Eye wash
First aid kit

Instructional Approach:

Lecture
Demonstration
Discussion

References:

Manual for Sanitary Chemistry and Sanitary Microbiology, Linn-Benton Community College, Carnegie & Wooley, 1975.

Standard Methods, 14th Edition

Wastewater Laboratory Procedures & Chemistry, from Operation of Wastewater Treatment Plants by Kerri.

Class Assignments:

Read handout

Module No:	Topic: Safety
Instructor Notes:	**Instructor Outline:**
	1. Identify, describe and demonstrate the use of
	a. Safety showers
Distinguish between A, B, & C class extinguishers. Show Soda Water CO_2, Dry chemical	b. Fire extinguisher
	c. Fire blanket
	d. Eye wash
Do not try to teach a first aid course in this module.	e. First aid kit with instruction sheet
Demonstrate top draw and bottom draw on a fume hood.	f. Fume hood
	2. a. Describe emergency situations and indicate proper actions.
Stress that no two emergency situations are the same.	b. Have participants indicate how the situation could be prevented and what actions must be taken after the incident.
Handout Laboratory Safety	3. Discuss personal protection equipment and the use of it.
	4. Discuss handout chemical hazards. Participant must be able to state the safety hazards and proper storage methods of Group A chemicals on handout.

LABORATORY SAFETY
Carnegie & Wooley
Manual for Sanitary Chemistry & Sanitary Microbiology
Linn Benton Community College
Albany, Oregon

Introduction

For the inexperienced and careless operator, the treatment plant laboratory can be extremely hazardous. The laboratory is not necessarily a dangerous place, however. Intelligent precautions and an understanding of proper techniques make the laboratory less dangerous than most other industries.

A number of hazardous materials and conditions do exist. Be aware of these dangers. Prevent accidents.

Personal Protection

1. Wear Safety Goggles or Eyeglasses

   Eyes must be protected from splashing chemicals and flying broken glass by wearing goggles at all times.

2. Wear Lab Coat or Apron and Protective Shoes

   Protect clothing and body from corrosive chemicals. Tennis shoes or sandals are not acceptable.

3. Know Location of Safety Equipment

   A first-aid cabinet, a fire extinguisher, a fire blanket and an eye-wash fountain should be available. Know exactly where they are located and how to use them.

4. Toxic Fumes

   Any test involving a dangerous or unpleasant volatile material should be performed in a hood or well-ventilated part of the laboratory.

5. Measuring Chemicals

Never handle chemicals with the hands. Always use a spatula. Do not drip liquid chemicals. Pour stock solutions into a small beaker, then into the graduate. Pipette from the beaker, not the stock solution bottle.

6. High Temperature

Use protective gloves or long handled tongs when using autoclave, hot plate, furnace or oven.

7. Broken Glassware

Discard or repair cracked or broken glassware immediately.

8. Electrical

Check all electrical equipment to see that it is properly wired and grounded.

9. Wash up

Always wash your hands after handling chemical containers and test apparatus.

10. Eating

Never use glassware for serving food. Always wash before eating or smoking. It is not good to eat in the laboratory at all.

11. Labels

Always label containers with name of material, concentration, date, and your initials. This will prevent accidents with acids, etc. in unlabeled beakers and also prevent use of wrong reagents in lab tests.

Corrosive Chemicals

1. Acids (Sulfuric, Hydrochloric, Nitric, Glacial Acetic
    a. Concentrated acids are extremely corrosive to everything, including skin. Use glass and polyethylene containers.
    b. In case of spills, immediately add large quantities of water to the area and neutralize with sodium bicarbonate. Then clean up the area.
    c. Contact with skin burns very quickly. Wash immediately with large quantities of water and neutralize with sodium bicarbonate.
    d. Dilute concentrated acid by adding the acid to the water, never the reverse.
    e. Always pipette with a rubber bulb.
    f. In general, do not mix strong acids with strong bases. If it is necessary to mix these solutions, do so very slowly, with mixing and cooling in cold tap water or ice water.
2. Bases (Sodium Hydroxide, Potassium Hydroxide, Ammonium Hydroxide)
    a. Concentrated bases are also extremely corrosive to skin and clothing. Use glass (with rubber stopper) and polyethylene containers. Do not use glass stoppered bottles.
    b. In case of spill, wash with large quantities of water and neutralize with saturated boric acid solution.
    c. Always pipette with rubber bulb.
3. Others
    a. Chlorine gas - Secure covers to prevent escape of vapor.
    b. Ferric chloride - Extremely corrosive to metals. Avoid contact with skin.

Toxic Chemicals

1. Avoid Ingestion or Inhalation
   a. Solids - Cyanides, chromium, cadmium.
   b. Liquids - Carbon tetrachloride, ammonium hydroxide, nitric acid, bromine, chlorine water, chloroform, carbon disulfide. Use in hood.
   c. Gases - Hydrogen sulfide, chlorine, ammonia, hydrochloric acid. Use in hood.
2. Most Chemicals Have Warnings and Antidotes on Their Labels. Read Them Before you Use the Chemical.

Explosive or Flammable Materials

1. Acetylene, hydrogen, carbon disulfide, benzene, ethyl ether, petroleum ether, acetone. Store the materials according to fire regulations.
2. Use in hood. Do not use near open flame of exposed heating element. Do not smoke near the chemicals. Use extreme caution during distillation. Do not distill to dryness.

Infectious Materials

Although it is highly unlikely that an operator would contract diseases by working in a treatment plant, the possibility does exist.

1. Sewage contains bacteria and viruses which can cause diseases. Some diseases are contracted through breaks in the skin. Keep wounds covered and if necessary, wear protective gloves.
2. Some are contracted through the digestive tract. The best protection is to wash thoroughly after performing tests to avoid transferring bacteria to mouth while eating.
3. Immunization is provided for many of the diseases. Operators are encouraged to take full advantage of this type of protection.

Module No:	**Module Title:** Basic Laboratory Skills
	Submodule Title: General Skills
Approx. Time: 30 Min.	**Topic:** Notebooks and Bench Sheets

Objectives:

Upon completion of this module, the participant should be able to:

1. Describe information to be included in a general lab notebook.
2. Describe the utility of a lab bench sheet.

Instructional Aids:

Handouts
1. Lab notebooks
2. Bench sheets

Instructional Approach:

Lecture

References:

None

Class Assignments:

Read handouts

Module No:	Topic: Notebooks and Bench Sheets
Instructor Notes:	Instructor Outline:
Handout Lab Notebooks	Discuss lab notebooks
	Why they are kept What must be in one
Handouts Bench sheets	Discuss the use of bench sheets and their relationship to lab notebooks.
	Discuss bench sheets examples.

INSTRUCTIONS FOR KEEPING LABORATORY NOTEBOOKS

1. Entries should be recorded in ink or ball-point pen by the person doing the work, on the same day the work was done. Such person should date the page at the beginning of each day's entry and should initial the page after each day's entry. Entries should be made on only one side of each page. The blank side facing each page may be used for calculations not constituting a material part of the information recorded.
2. Each new project should, as a first entry, include a clear, concise statement of what is to be done and what is hoped to be achieved. All entries should be made in such detail that anyone not directly associated with the work will be able to read and understand the scope and object of the work described.
3. Each page should be filled in completely either with written matter or diagonal lines before starting on the next page. No blanks should be left, for example, for later insertion of analyses.
4. No attempt should ever be made to correct or obliterate any entry. Necessary corrections or deletions should be made by drawing a single line through the portion to be deleted, being sure to leave the original matter legible. As required, substitute words may be written above the deleted matter. All such changes should be initialed and dated as of the date of correction. If possible, an explanation of the change should be made either in the margin or immediately following the correction if that portion of the page has not already been filled.

5. Any sketches or drawings which are not originally made on the notebook pages may be inserted but care should be taken that each page is appropriately identified by title and date. Reference should be made in the text of the notebook entry to such insertions and the date when such pages became available. This will refute any charge that such inserted pages were prepared at a date later than indicated.
6. Each person who has the duty of recording experiments should have his own notebook and should not permit others to make entries in it. In the case of shift work this rule may be relaxed if the records are otherwise adequately corroborated.
7. There is no objection to having separate notebooks for separate projects but care should be taken to insure that entries are made in chronological order and that there is sufficient identification of each entry to maintain continuity.

SOLIDS DETERMINATION

Percent Total Solids (T.S.)
and
Percent Volatile Solids (V.S.)

Source			
Dish No.			
Weight of Dish + Sample			
Weight of Dish			
Weight of Wet Sample			
Weight of Dish + Sample After Drying			
Weight of Dish			
Weight of Dry Sample			
% Total Solids			
Average T.S.		X	X
Weight of Dish + Sample After Ignition			
Weight of Dish			
Weight of Residue			
% Fixed Solids			
Average		X	X

Formulae:

1. $\frac{\text{Wt. of Dry Sample}}{\text{Wt. of Wet Sample}} \times 100 = \%$ Total Solids (T.S.)

2. $100 - \%$ Total Solids $= \%$ of Moisture

3. $\frac{\text{Wt. of Residue}}{\text{Wt. of Dry Sample}} \times 100 = \%$ Fixed Solids (F.S.)

4. $100 - \%$ Fixed Solids $= \%$ Volatile Solids (V.S.)

This method is usually used in sludge solids analysis.

Module No:	Module Title: Basic Laboratory Skills
	Submodule Title: General Skills
Approx. Time: 30 Min.	Topic: Labeling

Objectives:

Upon completion of this module, the participant should be able to:

1. Describe the necessity of proper labeling of chemical stock bottles, sample bottles, flasks etc.
2. State the information required on a chemical stock bottle.
3. State the information required on a sample bottle.

Instructional Aids:

Handout: "Labels"

Instructional Approach:

Lecture

References:

Self-monitoring Procedures, Basic Laboratory Skills, USEPA, Engel, Highby, Wagner.

Class Assignments:

Read handouts

Module No:	Topic: Labeling

Instructor Notes:	Instructor Outline:
	1. a. Discuss labeling of chemical stock bottles, sample bottles and flasks.
	b. Discuss dating of chemicals and reagents.
	c. Discuss sample labeling
Handout: Labels	2. List the data required on chemical stock bottle.
	3. List the data required on a sample bottle.

LABELS

Labeling

When a chemical or a piece of equipment is used for a specific analysis, it should have some type of identification. When you prepare a chemical from a stock container (purchased from a supply house), you must identify that chemical properly. The stock container will have all the necessary information on its label. A general format for labeling reagent bottles is as follows.

Chemical Name
Chemical Formula
Concentration

Date Initials

In preparing a chemical reagent a specific procedure would be as follows:

> Prepare a sulfuric acid solution 10% by volume by pouring 10 ml of concentrated sulfuric acid (H_2SO_4), into 90 ml of distilled water. Cool the solution to room temperature and transfer to a storage bottle.

The label should be:

Sulfuric Acid
H_2SO_4
10% by Volume

12/19/74 WTE

All necessary information has been included on the label to properly identify it. It takes a little more time but it is well worth it in the long run.

Several labeling tools are available, and each has its place in the laboratory. Most beakers and flasks will have a hexagon space of ground glass which can be used to identify it.

A lead pencil should be used for this type of marking.

Grease pencils are primarily used for test tubes. It should be noted that the grease pencil marking will readily rub off. When porcelain is labeled, a special technique should be used, since the item will be repeatedly heated and cooled. An etching device such as a Vibra-Groover, should first be used to put either a number or letter on the item. Next the etching should be filled in by rubbing it with a stick dipped in 1% Ferric Chloride ($FeCl_3$) solution (can either be prepared or commercially purchased). The porcelain crucible or other item is then placed in a muffle furnace (Approximately 600° C.) and fired for 10 minutes. After cooling the porcelain is ready for use. Whatever labeling techniques you use, be consistent, and remember that the label is intended not only for convenience but also for safety.

CHEMICAL NAME	POTASSIUM DICROMATE
SYMBOL	$K_2CR_2O_2$
CONCENTRATION	0.250 N
DATE	JULY 5, 1977
PREPARED BY	BY JOHN DOW

SAMPLE SITE	CHLORINE CONTACT TANK EFF.
TIME & DATE	11:30 A.M. - JULY 4, 1977
SAMPLE TYPE	GRAB SAMPLE
TYPE OF PRESERVATION	NO PRESERVATION
SAMPLER	BY JOHN DOW

Module No:

Module Title:

Submodule Title.

Approx. Time:

1 hour

Topic:

Sampling

Objectives:

1. Explain why sampling and preservation is just as important as the accuracy and precision of the analysis.
2. Differentiate between grab and composite sampling.
3. List three general methods of sample preservation.

Instructional Aids:

Handout: "Sampling"
Handout: Sample Preservation

Instructional Approach:

Lecture

References:

1. Kerri, Wastewater Laboratory Procedures
2. Standard Methods, 14th Edition
3. Methods for Chemical Analysis of Water and Wastewater, USEPA, Technology Transfer

Class Assignments:

Read handouts

Module No:	Topic: Sampling

Instructor Notes:	Instructor Outline:
Handout "Sampling"	1. a. Discuss sampling in general
	b. List the objectives of sampling
	c. Indicate that the analysis and results are only as go at the sample.
	2. a. Differentiate between grab and composite samples.
	b. List types of composite samples.
Handout "Sample Preservation"	3. a. Discuss methods of preservation
	1. Retard biological action 2. Retard chemical change 3. Reduce volatility

SAMPLING
Carnegie & Wooley
Manual for Sanitary Chemistry & Sanitary Microbiology
Linn Benton Community College
Albany, Oregon

The most neglected technique in laboratory control tests is in the collection and handling of samples. Even though a test is performed carefully and accurately, the result may be completely wrong and meaningless, unless a good representative sample is taken.

Cardinal Rules

The cardinal rules for sampling spell CAP:

1. CLEANLINESS - of all containers, including caps, and measuring devices that the sample comes in contact with.
2. ACCURACY - of records. The sample label should note the type of sample, source of sample, source, location of sampling point, the date and hour sampled, the temperature of the sample, and recent weather conditions.
3. PRESERVATION. Sewage samples contain living organisms which continue to grow unless the life processes are slowed by lowered temperatures or halted by addition of chemicals. Chemical degredation can also occur if samples are not properly preserved.

Principles of Sampling

1. The sample should be taken where the sewage is well mixed.
2. Large particles which may be in the sewage should be broken into smaller pieces or excluded.
3. No deposits, growths or floating materials that have accumulated at the sampling point should be included.

4. Samples should be tested as soon as possible.

Types of Samples

DEFINITION: A sample is a part of anything that is presented as evidence of the quality of the whole.

1. GRAB SAMPLES. Grab samples are taken because they are necessary or because there is a lack of time to catch composite samples. For some tests grab samples must be used. Tests such as residual chlorine, dissolved oxygen, and pH are determined from grab samples as a portion of sewage which cannot be mixed. For some tests grab samples can be used because the quality of the component to be sampled remains uniform for a period of a day or longer. An example is a digestor sample. A grab sample is simply one taken at a specific time with no regard to flow rate.
2. COMPOSITE SAMPLES. Composite samples are representative of the character of the sewage over a period of time. BOD, settleable solids and suspended solids tests are usually run on composite samples. The effects of intermittent changes in strength and flow are eliminated. The portion collected should be obtained with sufficient frequency to obtain average results. The rate of sewage flow must be measured when each portion is taken and the volume of the portion adjusted to the flow at the particular time of sample. Samples may be composited either by mechanical samplers or by hand. A composite sample is a series of grab samples poured together to make one sample.

Use the following formula to determine the volume of sample to be taken at each sampling interval to obtain a weighted composite sample.

$$\frac{\text{Total sample volume in ml}}{\text{No. of sampling time}} \times \frac{\text{Flow rate at sampling}}{\text{Average flow rate}} = \text{ml sample at sampling time}$$

Sample Preservation

Both grab and composite samples should be chilled to 3^{o} - 4^{o} C immediately. This is particularly true for BOD and all biological tests. Samples for certain tests may require some type of chemical preservative. It is not possible to preserve samples for other tests such as DO and temperature. The following table lists some common tests and preservation methods:

Preservation Methods

Test	Preservative	Maximum Holding Period
Acidity-Alkalinity	Refrigeration at 4^{o}C	24 hours
Biochemical Oxygen Demand	Refrigeration at 4^{o}C	6 hours
Chemical Oxygen Demand	2 ml H_2SO_4 per liter	7 hours
Chloride	None required	----
Color	Refrigeration at 4^{o}C	24 hours
Dissolved Oxygen	Determine on site	No holding
Hardness	None required	----
Nitrogen, Ammonia	40 mg $HgCl_2$ per liter - 4^{o}C	7 days
Nitrogen, Nitrate - Nitrite	40 mg $HgCl_2$ per liter - 4^{o}C	7 days

SAMPLE PRESERVATION

Complete and unequivocal preservation of samples, either domestic sewage, industrial wastes, or natural waters, is a practical impossibility. Regardless of the nature of the sample, complete stability for every constituent can never be achieved. At best, preservation techniques can only retard the chemical and biological changes that inevitably continue after the sample is removed from the parent source. The changes that take place in a sample are either chemical or biological. In the former case, certain changes occur in the chemical structure of the constituents that are a function of physical conditions. Metal cations may precipitate as hydroxides or form complexes with other constituents; cations or anions may may change valence states under certain reducing or oxidizing conditions; other constituents may dissolve or volatilize with the passage of time. Metal cations may also adsorb onto surfaces (glass, plastic, quartz, etc.), such as, iron and lead. Biological changes taking place in a sample may change the valence of an element or a radical to a different valence. Soluble constituents may be converted to organically bound materials in cell structures, or cell lysis may result in release of cellular material into solution. The well known nitrogen and phosphorus cycles are examples of biological influence on sample composition.

Methods of preservation are relatively limited and are intended generally to (1) retard biological action, (2) retard hydrolysis of chemical compounds and complexes and (3) reduce volatility of constituents.

Preservation methods are generally limited to pH control, chemical addition, refrigeration, and freezing. Table 1 shows the various preservatives that may be used to retard changes in samples.

Many water and waste samples are unstable. In situations where the interval between sample collection and analysis is long enough to produce changes in either the concentration or the physical state of the constituent to be measured, the preservation practices in Table II are recommended.

TABLE I

Preservative	Action	Applicable to:
$HgCl_2$	Bacterial Inhibitor	Nitrogen forms, Phosphorus forms
Acid (NHO_3)	Metals solvent, prevents precipitation	Metals
Acid (H_2SO_4)	Bacterial Inhibitor	Organic samples (COD, oil & grease organic carbon)
	Salt formation with organic bases	Ammonia, amines
Alkali (NaOH)	Salt formation with volatile compounds	Cyanides, organic acids
Refrigeration	Bacterial Inhibitor	Acidity-alkalinity, organic materials, BOD, color, odor, organic P, organic N, carbon, etc. Biological organism (coliform, etc.)

In summary, refrigeration at temperatures near freezing or below is the best preservation technique available, but it is not applicable to all types of samples.

The recommended choice of preservatives for various constituents is given in Table 2. These choices are based on the accompanying references and on information supplied by various Regional Analytical Quality Control Coordinators.

TABLE 2

RECOMMENDATION FOR SAMPLING AND PRESERVATION OF SAMPLES ACCORDING TO MEASUREMENT (1)

Measurement	Vol. Req. (ml)	Container (2)	Preservative	Holding Time (6)
Acidity	100	P, G	Cool, 4° C.	24 Hrs.
Alkalinity	100	P, G	Cool, 4° C.	24 Hrs.
BOD	1000	P, G	Cool, 4° C.	6 Hrs.
COD	50	[illegible], G	H_2SO_4 to pH 2	7 Days
Dissolved Oxygen Probe	300	G only	Det. on site	No Holding
Winkler	300	G only	Fix on site	No Holding
Nitrogen				
Ammonia	400	P, G	Cool, 4° C. H_2SO_4 to pH 2	24 Hrs. (4)
Kjeldahl	500	P, G	Cool, 4° H_2SO_4 to pH 2	24 Hrs. (4)
Nitrite	50	P, G	Cool, 4° C.	24 Hrs. (4)
Oil & Grease	1000	G only	Cool, 4° C. H_2SO_4 to pH 2	24 Hrs.
pH	25	P, G	Cool, 4° C. Det. on site	6 Hrs. (3)
Residue				

TABLE 2 Cont.

Measurement	Vol. Req. (ml)	Container (2)	Preservative	Holding Time (6)
Filterable	100	P, G	Cool, 4° C.	7 Days
Non-Filterable	100	P, G	Cool, 4° C.	7 Days
Total	100	P, G	Cool, 4° C.	7 Days
Volatile	100	P, G	Cool, 4° C.	7 Days
Settleable Matter	1000	P, G	None Req.	24 Hrs.
Specific Conductance	100	P, G	Cool, 4° C.	24 Hrs.
Temperature	1000	P, G	Det. on site	No Holding
Turbidity	100	P, G	Cool, 4° C.	7 Days

1. More specific instructions for preservation and sampling are found with each procedure as detailed in this manual. A general discussion on sampling water and industrial wastewater may be found in ASTM, Part 23, p. 72 - 91 (1973).
2. Plastic or glass
3. If samples cannot be returned to the laboratory in less than 6 hours and holding time exceeds this limit, the final reported data should indicate the actual holding time.
4. Mercuric chloride may be used as an alternate preservative at a concentration of 40 mg/l, especially if a longer holding time is required. However, the use of mercuric chloride is discouraged whenever possible.

5. If the sample is stabilized by cooling, it should be warmed to 25° C. for reading, or temperature correction made and results reported at 25° C.
6. It has been shown that samples properly preserved may be held for extended periods beyond the recommended holding time.

Module No:

Module Title:
Basic Laboratory Skills

Submodule Title:
General Skills

Approx. Time:
2 hours

Topic:
General Lab Equipment and Glassware

Objectives:

Upon completion of this module, the participant should be able to:

1. Identify and operate the following lab equipment: Vacuum pump, lab burner fume hood, lab oven, dessicator, hot plate, stirrer
2. Identify the following lab glassware: Buret, pipet (volumetric), pipet (mohr), graduated cylinder, Erlenmeyer flask, vacuum flask, volumetric flask, separating funnel, buchner funnel, gooch crucible, watch glass, beaker, Walter crucible holder, buret clamp.
3. Demonstrate proper methods of glassware cleaning and indicate when special cleaning reagents are needed.

Instructional Aids:

Lab equipment per handout
Handouts
1. Laboratory Equipment Description and Use
2. Glassware cleaning

Instructional Approach:

Lecture
Demonstration

References:

Manual for Sanitary Chemistry and Sanitary Microbiology, Carnegie & Wooley.

Class Assignments:

Read handouts

Module No:	Topic: General Lab Equipment and Glassware

Instructor Notes:	Instructor Outline:
	1. Demonstrate the use of: a. Vacuum pump b. Lab burners c. Fume hoods d. Lab ovens e. Dessicators f. Hot plates g. Magnetic stirrers
Proper use of equipment will be covered in following topics.	2. Identify and demonstrate the proper handling and storage of:
Handout Laboratory equipment description and use.	Burets Pipets Volumetric Mohr Graduated cylinders Erlenmeyer flasks Vacuum flasks Volumetric flasks Separatory funnel Buchner funnel Gooch crucible Watch glass Beaker Walter crucible holder Buret clamp
Handout Glassware cleaning	3. Discuss handout on glassware cleaning Note safety precautions for use of strong acid and strong base cleaning solutions.

LABORATORY EQUIPMENT
DESCRIPTION AND USE

The well equipped treatment plant lab should have the necessary equipment and glassware to perform all necessary tests, some of which will be run simultaneously. In addition, the lab must have the necessary supporting equipment to make up solutions and perform other routine lab tasks. The following items should be considered minimum for an efficient and smoothly operating treatment plant laboratory:

(Mention of any piece of equipment by brand name does not necessarily mean endorsement of that brand by Linn-Benton Community College or the Environmental Protection Agency, but is used for illustrative purposes only.)

1. BALANCES

a. BEAM BALANCE

This balance should have a capacity of 500 g. and a precision of 0.02 g. This balance is used for quick measurements, such as weighing chemicals for the preparation of most solutions. Detailed instructions for the operation of the balance accompany the instrument. Read them thoroughly before attempting to make measurements. In general, treat the instrument gently and keep it clean.

b. ANALYTICAL BALANCE

This balance should have a capacity of 160 g. and a precision of 0.1 mg. This balance is used primarily for solids determinations and for weighing dry chemicals in preparation of standard solutions. Detailed instructions for operation also accompany this instrument. Strict adherence to the directions is necessary to avoid damage. This instrument is extremely sensitive and cannot be jarred or treated roughly. Keep it clean, inside and out.

2. pH EQUIPMENT

a. pH METER

The pH meter should have a range of 0 to 14 pH, and deliver ± 0.1 pH accuracy. This instrument is used to adjust pH of solutions, titrations, and other procedures requiring some degree of accuracy. Detailed instructions for the operation of the pH meter are included in another module.

b. pH TEST PAPER

pH test paper is a convenient tool for getting a rough check of the pH very quickly. It can be obtained in nearly any range. For general use, a range of 1 to 11 pH and an accuracy of 0.5 pH is adequate.

The test paper is treated with an indicator which will change color when moistened. Distinct color changes occur over the entire range of pH. To determine the pH of a solution with pH test paper, obtain a drop solution with a clean Pasteur pipette or stirring rod and apply it to a piece of the test paper. The paper will change color immediately. Determine the pH by comparing the color chart on the dispenser.

3. INCUBATORS

a. BOD INCUBATOR

BOD's are incubated at 20° C. and normally a relatively large number of bottles are used. Therefore a large cabinet type incubator that will hold several hundred BOD bottles with a sensitivity of ± 0.5° C. is required.

b. BENCH MODEL INCUBATOR

Most bacterial tests are run at 35° C. Therefore, an additional incubator is needed. The incubator should be large enough to accommodate the maximum number of plates which would ever be handled at the same time. Sensitivity should be at least ± 0.2° C.

4. WATERBATH

a. CONTROLLED TEMPERATURE

A water bath at 45° C. is required for fecal coliform membrane filter test. The bath must be large enough to accommodate several plastic bags containing membrane filter dishes. This bath should have a sensitivity of at least ± 0.2° C. and a range from room temperature to 100° C. Several other tests require water baths at different temperatures. Often the same bath can be used, but it must be easily adjusted between tests.

b. STEAM TABLE

The solids tests require a steam table for evaporation of the sample. Often the controlled water bath can double as a steam table if it can be covered properly and still allow the evaporating dish to sit down into the bath. In larger plants, it would be advisable to have a separate steam table, since many of the tests will overlap. The steam table must reach 100^{0} C. and have an automatic overflow water level control.

5. MICROSCOPES

a. COMPOUND MICROSCOPE

A microscope is required for observation of sludge samples and bacteria. The compound microscope should have at least three objective lenses; a low power (10X), high dry power (43X), and an oil immersion lens. An electrical light source is recommended. Do not attempt to operate the compound microscope without direct, personal instruction from some one experienced with your particular model.

b. DISSECTING MICROSCOPE

The binocular dissecting microscope is quite helpful in properly identifying coliform colonies in the membrane filter tests. The microscope should have a range of 1X-3X with an electric lamp light source.

6. STIRRING HOT PLATE

The stirring-hot plate is used in the preparation of solutions, as well as in several tests. The heating and stirring units should be able to be operated separately or together. The plate should heat from 150 to 700° F. and the stirrer should run from 0 to 1800 rpm.

7. CENTRIFUGE

A small bench top centrifuge is used to clarify some wastewater samples. The instrument should have the capacity to hold 8, 15 ml. or 4, 50 ml. conical centrifuge tubes and run at speeds up to 3200 rpm. A timer is convenient. Caution must be taken in the operations of any centrifuge to be sure the load is balanced. Tubes opposite one another must be the same weight. The weight can be checked on a balance or by leveling the amount of liquid in the two tubes. If only one tube is needed for your samples, make a balance tube up with water. Do not operate the centrifuge with the lid up. Accelerate the centrifuge slowly to avoid undue strain on the motor. Clean up any spills in the instrument immediately.

8. SPECTROPHOTOMETER

The spectrophotometer is required for color intensity determinations on several tests. The instrument should have the capacity to work in the ragne of 400-700 mm. Detailed instructions for the operation of the spectrophotometer are included in another section.

9. MUFFLE FURNACE

The muffle furnace is used in the volatile solids tests and must reach a temperature of 600° C. and space enough to handle three or four evaporating dishes is recommended. Use extreme caution when working around the oven. Always wear insulated gloves and use long handled tongs to insert and remove dishes.

10. DRYING OVEN

The drying oven is used to dry crucibles, dishes, filter paper, chemicals, and glassware. It should have a heating capacity of up to 150° C. and control sensitivity of ± 0.5° C. Use caution because of heat. Handle material with tongs or gloves.

11. AUTOCLAVE

The autoclave is used for sterilizing solutions, bacterial growth media, and glassware. It must have the capacity to develop and hold 15 psi at 121° C. for any length of time. Size is not important as long as it is large enough to accommodate the volume of work required. Bench top sterilizers are satisfactory as long as they meet the above requirements. Each autoclave is slightly different. Operating instructions are included with the instrument and should be read prior to operation. Preferably, do not operate without the instruction of someone familiar with the operation of your particular model. Use caution since the autoclave develops high pressures and high temperatures. Always remove hot items with tongs or gloves.

12. WATERSTILL

Distilled water is required in nearly every test performed in the laboratory. High quality distilled water can be obtained from several commercial models. In selecting the still for your lab, determine the quantity of water needed for operation. For most laboratories, a capacity of 2 gal/hour is satisfactory. Directions for operation accompany the still. Of critical importance is not allowing the still to run dry. Some laboratories find it desirable to also process their distilled water through a demineralizer to obtain ultra-pure water. Although this is not required, it is recommended for several tests.

13. BUNSEN BURNER

The Bunsen burner is used as a source of heat for boiling and to sterilize equipment during biological transfers. The burner should be compatible with the type of gas available for fuel. Self-containers gas-cylinder units are available if commercial gas lines are not present.

14. DESICATTOR

The desicattor is used to store items that must not take moisture from the atmosphere. They should be large enough to hold several evaporation dishes.

15. ASPIRATOR

A vacuum pump or aspirator on the sink faucet is needed for several filtration steps. The aspirator can be connected directly to the cold water tap. The vacuum hose should run to a "water-trap" before it is connected to the vacuum flask to prevent water from surging up into the flask where the vacuum is released.

16. BURETS

a. PRECISION BURETS

The buret is essential for several treatment plant tests. It is designed to deliver liquids in a controlled fashion, such that additions can be made dropwise or intermittently and the final volume delivered determined. The straight bore, and Teflon stopcock is recommended for general use. Burets with larger or smaller capacities can be obtained. Fill the buret by adding the liquid with aid of a funnel to the top with the stopcock closed. Fill it well above the 0 ml. mark. Then bleed out the tip so that the orifice through the stopcock and the tip are free of air bubbles. Continue bleeding until the meniscus at the top of the buret reads 0 ml. Dispense the liquid by grasping the stopcock with the left hand, leaving the right hand free to agitate the flask below. After the required volume has been dispensed, read the meniscus. Notice that the values increase from top to bottom. The difference between the final buret reading and the initial buret reading will give the exact volume dispensed. By this method, it is not necessary to refill between each operation. Simply calculate the difference in buret reading as you continue to dispense the liquid. However, be careful not to dispense below the 50 ml. mark.

17. PIPETTES

a. MEASURING PIPETTES

Measuring pipettes are used for a variety of purposes. They can be obtained in capacity from 0.1 ml. to 20 ml. with different subdivisions. Every lab should have a selection of pipettes from 0.1 ml to 20 ml., mostly 1, 5, and 10 ml. volume. Measuring pipettes come in two types; those calibrated clear to the tip and those not calibrated to the tip. The first type is referred to as a "blow-out" pipette, since it is necessary to force the last drop out of the tip in order to deliver the measure volume. The second type is operated on the same principle as a buret. The liquid is drawn up into the pipette and the desired volume delivered by allowing the liquid to drain out, using the meniscus as the indicator of volume delivered.

The liquid can be drawn into the pipette by mouth or by a rubber bulb. In general, it is advisable to use a rubber bulb. Experienced lab technicians may find it more efficient to pipette by mouth, however, never pipette strong acids or bases, toxic solutions, sewage samples, or bacterial cultures by mouth.

b. VOLUMETRIC PIPETTS (TRANSFER PIPETTES)

Volumetric pipettes are designed to give the greatest accuracy in pipettes. They will deliver only one volume and range in capacity from 1 ml. to 50 ml. Each lab should have a supply of 1, 5, 10, 20 and 50 ml. volumetric pipettes. Their operation is identical to the measuring pipettes except that even though they are designed to deliver clear to the tip, they are NOT the blow-out type. They are calibrated to deliver the prescribed volume by simply touching the tip to the side of the container for a few seconds. The small drop remaining in the pipette is not included in the prescribed volume of the pipette.

c. TRANSFER PIPETTES (EYE-DROPPERS)

Transfer pipettes, commonly called eye-droppers, are useful in delivering small unmeasured quantities of liquid such as adding acid to adjust pH in the pH meter. They are operated by using a small rubber bulb to take up and dispense the liquid.

18. FLASKS

a. ERLENMEYER FLASK

The Erlenmeyer flask is a general purpose flask used for containing and mixing solutions. They range in capacity from 10 ml. to several liters.

b. FILTER FLASK

The vacuum filter flask is essentially an Erlenmeyer flask with a side-arm attachment to receive a vacuum hose. Filtration is accomplished by placing a filter funnel in the neck of the flask and drawing the liquid through with the aid of the vacuum.

c. VOLUMETRIC FLASK

The volumetric flask is designed to accurately measure large volumes of liquid, primarily in the preparation of reagents and standard solutions. They range in size from 1 ml. to 2000 ml. The 50, 100, 500, and 1000 ml. sizes are recommended for general lab use. The volumetric flask is calibrated to contain the prescribed volume, not to deliver. The stoppered variety is more convenient for use in solution preparation.

19. GRADUATED CYLINDERS

Graduated cylinders, or graduated, are used to measure large volumes of liquid and are calibrated "to deliver" not "to contain". That means, if the graduate is filled and the contents poured out, it will deliver the prescribed volume. The drops left behind are not included in the prescribed volume. It is considered volumetric but does not have the accuracy of the volumetric flask. Graduates range from 5 ml. to 2000 ml. Sizes of 10, 50, 100, 200, 500, 1000 ml. are recommended for general lab use.

20. BEAKERS

Beakers are the most common non-volumetric piece of glassware and range in size from 1 ml. to 4000 ml. Sizes of 50, 150, 250, 600, 1000, and 2000 ml. are recommended for general lab use. Although they do have graduations, they should not be used to measure accurate volumes.

21. BOTTLES

a. PLASTIC BOTTLE

Polyethylene bottles are convenient to use for chemical storage. Such bottles can be used to collect and transfer sewage samples. Dark colored plastic bottles protect light sensitive chemicals. High temperature polyethylene can be sterilized by autoclaving. Sizes from 1 oz. to several gallons are available in a variety of designs.

b. GLASS STOPPERED BOTTLES

Glass stoppered bottles are ideal for strong acid solutions, and many other reagents. However, strong bases tend to "freeze" the stoppers. Rubber stoppers should be used for strong bases. Glass stoppered bottles range in size from 30 to 2000 ml.

c. MILK DILUTION BOTTLES

Dilution bottles are 125 ml. volume glass bottles with one calibration at 99 ml. They are used for bacterial and sewage dilutions and can be autoclaved.

d. DROPPING BOTTLES

Dropping bottles with hooded glass stopper or small eye droppers attached are recommended for use with stains and indicators.

e. SQUEEZE BOTTLES

Plastic squeeze bottles are used to dispense distilled water during rinsing operations.

f. CARBOYS

Large plastic carboys, from 2-12 gallon capacity, with spigots, are recommended for storage of distilled water, buffered water, and dilution water.

22. EVAPORATING DISHES

Porcelain evaporating dishes are used to dry chemicals and sewage samples. Sizes of 70 and 150 ml. capacity are recommended. Sufficient number to handle several samples each day should be on hand.

23. GOOCH CRUCIBLE

The Gooch crucible is used in solids determination. The 35 ml. size is recommended. The illustration shows the crucible in the rubber-adapter for filtering flask.

24. IMHOFF CONE

The one liter volume with blunt tip for raw sewage and sharp tip for final sewage is used for settleable solids determinations.

25. BUCHNER FUNNELS

Porcelain Buchner funnels are used in solids determinations. The 80 mm. and 115 mm. diameter sizes would be recommended.

26. TONGS

a. CRUCIBLE TONGS

Both the normal 9" and long 20" tongs are recommended.

b. EVAPORATING DISH TONGS

Stainless steel safety dish tongs are best for handling hot evaporating dishes.

c. BEAKER TONGS

For hot beakers and other similar objects, the Safety Beaker clamp is recommended.

d. FLASK TONGS

In addition, the Safety Flask Clamp is recommended.

27. BACTERIOLOGICAL EQUIPMENT

a. PETRI DISHES

Either glass or disposable plastic petri dishes are acceptable. For the membrane filter procedures, the 60 x 15 mm. size is recommended. The 100 x 20 mm. size is used for total plate count and wherever agar plates are required.

b. MEMBRANE FILTRATION APPARATUS

A stainless steel or glass funnel, with base and filter support screen for 47 mm. diameter membrane filters is recommended. The whole apparatus must be able to withstand autoclaving.

d. WIRE LOOPS

A platinum wire loop with a 3 mm. loop is used for bacteriological transfers. A wooden or aluminum handle is acceptable.

28. FILTER PAPER

a. STANDARD FILTER PAPER

A high grade, medium weight, rapid filtering paper comparable to Whatman No. 1 is required for several tests. It is recommended to have a selection of sizes (7, 11, & 24 cm.) on hand.

b. MEMBRANE FILTERS

Sterile membrane filters with sterile absorbent pads are required for the membrane filter tests. The filters should be 47 mm. in diameter, 0.45 ~~mm~~ um. pore size, white with grid.

c. GLASS FIBER FILTERS

Ultra-fine filter, which retains particles in the semi-colloidal range with a thickness of 0.26 mm. and a diameter of 2.4 cm. is required for the suspended solids test. Filters equivalent to Whatman Grade GF/C is acceptable.

29. MISCELLANEOUS ACCESSORIES

a. RUBBER STOPPERS
b. CORK STOPPERS
c. RUBBER TUBING
d. TYGON TUBING
e. VACUUM TUBING
f. RING STANDS
g. RINGS & FUNNEL SUPPORTS
h. CLAY TRIANGLE
i. HOSE CLAMPS
j. ASBESTOS PAD

k. SPATULA

l. FORCEPS

m. PIPETTE FILLER (BULB)

n. CRUCIBLE HOLDER

o. ASBESTOS GLOVES

p. PIPETTE WASHER

GLASSWARE CLEANING METHODS

Carnegie & Wooley
Manual for Sanitary Chemistry & Sanitary Microbiology
Linn Benton Community College
Albany, Oregon

Clean glassware is essential to performing meaningful tests. Normally it is easiest to clean immediately after use, since materials will dry and stick to the glass if left for a period of time. If stored in a closed shelf it will not generally be necessary to wash again before use, however for extremely sensitive tests a distilled water rinse would be advisable before use.

Cleaning Solutions

1. Chromic Acid
    - A. Dissolve approximately 60 g of potassium dichromate in hot water.
    - B. Slowly add enough concentrated sulfuric acid to make one liter. Commercial preparations of this mixture are available from several chemical supply houses.
2. Hot Detergent

    Laboratory detergents are available in several forms. To avoid excess sudsing, use sparingly.

Cleaning Methods

1. Stopcock Grease (Petroleum Base)
    - A. Dissolve grease in acetone.
    - B. Wash with detergent.
    - C. Rinse with tap water four times.
    - D. Rinse with distilled water three times.

2. Stopcock Grease (Silicone Base)
    A. Soak for one half to two hours in sulfuric acid.
    B. Rinse with acetone.
    C. Wash with detergent.
    D. Rinse with tap water four times.
    E. Rinse with distilled water three times.
3. Bacteriological Contamination
    A. Soak in chromic acid mixture.
    B. Rinse with tap water 6 - 10 times
    C. Rinse with distilled water three times.
4. Fat and Oil Contamination
    A. Soak in chromic acid mixture.
    B. Rinse with tap water four times.
    C. Rinse with distilled water three times.
5. Organic Material
    A. Soak in chromic acid mixture.
    B. Rinse with tap water four times.
    C. Rinse with distilled water three times.

The rinsing operation must always be carried out thoroughly. Trace amounts of metal ions that remain due to carelessness may seriously affect organism growth and testing procedures. If an automatic dishwasher is used, glassware should still be given a thorough distilled water rinse before drying. Glassware may be dried at 103° C.

The cleaning operation is usually simplified if the pipettes, beakers, graduated cylinders, test tubes and flasks are immediately placed in a detergent solution after use. Delicate (and expensive) spectrophotometer cuvettes must be handled with extreme care and never exposed to the harsher cleaning agents.

In certain tests, such as the phosphate determination, special glassware cleaning techniques must be used. Special instruction will be included in the specific section dealing with that test.

Module No:	**Module Title:** Basic Laboratory Skills
	Submodule Title: General Skills
Approx. Time: 1 hour	**Topic:** Chemical Names and Formulas

Objectives:

Upon completion of this module, the participant should be able to:

1. Identify and choose the correct chemicals necessary for an analysis by name and formula given a set of lab chemicals and a list of chemicals required for an analysis.

Instructional Aids:

Handout: Names of Formulas and Compounds

Instructional Approach:

Lecture
Discussion

References:

Basic Lab Skills, Engel Highby Wagner

Class Assignments:

Read handoug
Worksheets in handout

Module No:

Topic:
Chemical Names and Formulas

Instructor Notes:	Instructor Outline:
Handout and Worksheets Names and Formulas of Compounds	1. a. Discuss very basic chemical nomenclature. b. Emphasize matching the exact name and formula with the chemical. c. Give examples of common errors in chemical selection by name or formula.

NAMES OF FORMULAS AND COMPOUNDS

In virtually every chemical analysis, the name and formulas of chemical compounds appear. Compounds are pure substances that are composed of two or more elements. Elements may be referred to as the basic building blocks of all substances. At present there are 105 elements known. These elements are shown in the periodic table.

Each element has a particular symbol. The symbol is an abbreviation for that element. The elements numbered (located above the symbol) 1 through 92 occur naturally (i.e. can be found in earth's crust, water or the atmosphere). Elements numbering 93-105 do not occur naturally but have been synthesized in small quantities in the laboratory. The symbols that are used to represent the elements are also used to represent compounds. For example the compound NaCl represents the combination of sodium (Na) (#11) and chlorine (Cl #17) and its name is sodium chloride.

All the chemical procedures that are included in this course will always refer to a compound with its formula and name together. For example: Prepare a 10% by volume sulfuric acid (H_2SO_4) solution by . . . Weigh out 186.15 grams of sodium thiosulfate ($Na_2S_2O_3$) . . . In several of the chemical formulas, you will note that subscripts are used. The subscript tells us how many atoms of that element are contained in the compound. In water (H_2O) there are two atoms of hydrogen and one atom of oxygen. The subscripts help to differentiate one compound from another. The compound hydrogen peroxide (H_2O_2) although similar to water is obviously not the same since there are 2 atoms of oxygen in the peroxide and only 1 atom in the water.

In choosing the proper chemical for an analysis, it cannot be overemphasized that the name and formula that occur on the label of the chemical must match the name and formula in the procedure that has been given. Several names may appear to be correct because of similarities in spelling such as:

sodium sulfate Na_2SO_4 and
sodium sulfite Na_2SO_3

These are not the same. The sulfate compound has one more oxygen atom than the sulfite. Another minor spelling variation would be potassium nitrate KNO_3 and potassium nitrite KNO_2. What is the difference here?

Another variation and in fact a very important property of compounds is the addition of the word anhydrous to the name. This means without water. The chemical has been prepared (at the factory) without water. If the chemical does have water in it, it will be referred to as hydrate.

Examples

Sodium Thiosulfate Pentahydrate ($Na_2S_2O_3.5H_2O$)

This means that the compound has 5 water molecules associated with it. Note that the prefixes to the word hydrate are mono, di, tri, tetra, penta, hexa, hepta, octa, nona, and deca referring to the numbers 1 through 10 respectively.

Calcium Chloride, Anhydrous ($CaCl_2$)

This means that the compound contains no water.

When choosing a chemical for a particular analysis, the stock chemical bottle must be studied very carefully. It contains a label that gives the name of the compound as well as the formula. It also contains

(CAUTIONS) such as explosive, toxic (poisonous). The hazards presented by these chemicals are not evident from appearance, smell, or everyday knowledge. Hazards must be foreseen and avoided. It is safest to assume that all chemicals, even water if not safely handled, can be hazardous. Read the label completely and follow the warnings that are indicated. The label will also mention any additional storage requirements that might be necessary for a particular reagent such as (Store at 25° C). The purity of the chemical is also indicated. Analytical or Reagent Grade is the highest purity. The amounts of impurities are shown on the label. The word ACS (American Chemical Society) also might be shown. This also means reagent grade. A lower grade of chemical would be laboratory or practical grade. Usually, amounts of impurities would not be listed on this label. A sample label is shown below.

$Na_2S_2O_3.5H_2O$ 5 lbs. CAUTION!!!

SODIUM THIOSULFATE
(crystals)

Reagent, A. C. S.

Emits Toxic Fumes When Heated
Keep container tightly closed.
Do not take internally.

The exercises on the following pages consist of various check lists and consumable supply lists. For every check list there is a consumable supply list. Complete these as the directions state.

Consumable Supplies I

1. 480 g. manganous sulfate tetrahydrate, $MnSO_4.4H_2O$
2. 500 g. sodium hydroxide, NaOH
3. 125 g. sodium iodide, NaI
4. 10 g. sodium azide, NaN_3
5. 4 plastic weighing boats
6. 1 small size spatula
7. 1 medium size spatula
8. 10 g. soluble starch
9. 10 ml chloroform
10. 186.15 g. sodium thiosulfate pentahydrate, $Na_2S_2O_3.5H_2O$
11. 6 g. potassium biiodate (or potassium biniodate) $KH(IO_3)_2$
12. 3 g. potassium iodide, KI
13. 10 ml concentrated sulfuric acid, H_2SO_4
14. Pen or pencil
15. Paper (to record data)

Check List - I

Chemical Names:

Place number from "consumable" list I by matching name.

_____a. Sodium Nitrate

_____b. Sodium Thiosulfate, Anhydrous

_____c. Sodium Thiosulfate Pentahydrate

_____d. Carbon Tetrachloride

_____e. Manganese Hydroxide

_____f. Manganous Sulfate Tetrahydrate

_____g. Magnesium Sulfate Heptahydrate

_____h. Potassium Bichromate

_____i. Sodium Iodide

_____j. Sodium Fluoride

_____k. Potassium Biiodate

_____l. Sodium Sulfite

_____m. Sodium Thiosulfite

_____n. Dilute Sulfuric Acid

_____o. Sodium Azide

_____p. Sodium Acetate

_____q. Concentrated Sulfuric Acid

_____r. Soluble Starch

Consumable Supplies - II

1. Small wad of cotton
2. 10 g. potassium dehydrogen phosphate, KH_2PO_4
3. 25 g. dipotassium hydrogen phosphate, K_2HPO_4
4. 35 g. disodium hydrogen phosphate heptahydrate, $Na_2HPO_4.7H_2O$
5. 3 g. ammonium chloride, NH_4Cl
6. 25 g. magnesium sulfate heptahydrate, $MgSO_4.7H_2O$
7. 30 g. anhydrous calcium chloride $CaCl_2$
8. 1 g. ferric chloride, $FeCl_3$
9. Manganous sulfate solution*, alkaline iodide azide solution*, starch solution*, standard sodium thiosulfate solution*, and concentrated sulfuric acid*.
10. Pen or pencil
11. Paper (for recording data)
12. Grease pencil

* Listed in the EMP on the Winkler Determination of Dissolved Oxygen azide modification.

Check List - II

Chemical Names:

Place number from "consumable" list by matching name.

_____a. Calcium Chloride Dihydrate

_____b. Sodium Chloride

_____c. Ammonium Chloride

_____d. Ferrous Chloride

_____e. Potassium Dihydrogen Phosphate

_____f. Magnesium Sulfate Heptahydrate

_____g. Ammonium Chlorate

_____h. Calcium Chloride, Anhydrous

_____i. Ferric Chloride

_____j. Dipotassium Hydrogen Phosphate

Consumable Supplies - III

1. 721.8 mg anhydrous potassium nitrate, KNO_3
2. 5.0 g sodium arsenite, $NaAsO_2$
3. 1 g. brucine sulfate, $(C_{23}H_{26}N_2O_4)_2.H_2SO_4 7H_2O$
4. 0.1 g. sulfanilic acid, $NH_2C_6H_4SO_3H.H_2O$
5. 3 ml concentrated hydrochloric acid, HCl
6. 500 ml concentrated sulfuric acid, H_2SO_4
7. 300 g. sodium chloride, $NaCl$

Check List - III

Chemical Formulae:

Place the number from the "consumable" list by the matching formula.

_____a. KNO_2

_____b. KCl

_____c. HCl

_____d. KNO_3

_____e. $NaClO_3$

_____f. $(C_{24}H_{28}N_2O_4)_2.H_2SO_4.7H_2O$

_____g. $NaAsO_2$

_____h. $(C_{23}H_{26}N_2O_4)_2.H_2SO_4.7H_2O$

_____i. H_3PO_4

_____j. $HN_2C_6H_4SO_3H.H_2O$

_____k. H_2SO_4

_____l. $NaClO$

_____m. $NaCl$

Module No:

Module Title:
Basic Laboratory Skills

Submodule Title:
General Skills

Approx. Time:
1 hour

Topic:
Matter

Objectives:

Upon completion of this module, the participant should be able to:

1. Note and observe volume change of liquids as temperature changes.
2. Note and observe hygroscopic properties of substances.

Instructional Aids:

Dry right, balance, hotplate, beaker, pipet

Instructional Approach:

Demonstration

References:

None

Class Assignments:

None

Module No: –	Topic: Matter

Instructor Notes:	Instructor Outline:
Start with cold water. Warm to not more than 50° C.	Demonstrate volume change of water and change in temperature.
	Demonstrate hydroscopic properties of NaOH and dry-rite using a balance.
	Weigh some dry-rite from a desiccator. Let set and weigh a second time. Note weight change.
	Discuss how the above two properties of matter affect accurate measurement.

Module No:

Module Title:
Basic Laboratory Skills

Submodule Title:
General Skills

Approx. Time:
1 hour

Topic:
Solutions

Objectives:

Upon completion of this module, the participant should be able to:

1. Calculate the concentration of a solution in mg/l or ppm. given the weight of solute in grams or milligrams and the volume of the solvent in liters.
2. Calculate the percent by weight of a solute given the weight of solute and volume of solution.
3. Recognize the letter N and M following numbers as indicating that the number is describing concentration.

Instructional Aids:

Handout "Solutions"

Instructional Approach:

Lecture

References:

Basic Lab Skills, Engel Highby Wagner

Class Assignments:

Read handout

Module No:	Topic:
—	Solutions

Instructor Notes:	Instructor Outline:
Handout "Solutions"	1. a. Discuss and demonstrate mg/l concentration calculations. b. Indicate the relationship between ppm and mg/l 2. a. Discuss and demonstrate percent by weight calculations. 3. a. Discuss molarity and normality as forms of chemical concentration measurement.

SOLUTIONS

Wastewater is a complex combination of water, floating and settleable solids, and dissolved solids. It is possible to separate the components of wastewater by physical and mechanical processes such as screening, settling, filtration and evaporation. Since this is the case, the chemist calls wastewater a mixture.

Let us take a sample of raw wastewater and run it through a very fine filter. All the floating and settleable solids will be removed. The filter also removes the turbidity. The filtrate, the liquid which comes through the filter, is a part of the original mixture. It contains water and dissolved solids. This clear liquid could be separated into two more components by distilling off the water. The dissolved solids would be left behind. Thus the filtrate, too, is a mixture. But it is a very special mixture called a solution. The term solution refers to a homogeneous mixture of two or more substances. The molecules of these substances are evenly distributed among one another. Because we cannot see any one component. A solution appears to be one pure substance. The components of a solution will not separate by settling.

The subject of solutions has been introduced by looking at wastewater because it is a mixture known to most of you. However, there are many other solutions which are familiar to you. We will now use some common solutions to continue our study of this important topic.

Chemist classify solutions into three major groups:

1. Gaseous solutions
2. Liquid solutions
3. Solid solutions

We will look at each group separately.

Gaseous solutions are made by mixing one gas in another. Air is a gaseous solution. Air is made of nitrogen, oxygen, argon, carbon dioxide and very small amounts of other gases. The molecules of each gas mix evenly to make a homogeneous mixture called air. The molecules of carbon dioxide are heavier than the molecules of the other gases but they do not settle out. We know that the amount of oxygen in a sample of air can change. There is less oxygen in a sample from the top of a high mountain than there is in a sample taken at sea level. Therefore, we must add to our description of a solution this fact:

The composition of a solution is changeable.

Liquid solutions are made by dissolving a gas, liquid or a solid in a liquid. Tap water is a solution which contains dissolved oxygen. The oxygen molecules are mixed uniformly with the water molecules to make a homogeneous mixture. The oxygen molecules do not settle out if the mixture is allowed to stand undisturbed. "Old Granddad" is an example of a liquid dissolved in another liquid. The alcohol molecules are dissolved uniformly in the water. We know this because every jigger tastes the same. The components of "Old Granddad" do not separate by settling. A sugar-water solution is an example of a solid dissolved in a liquid. The sugar crystals break up into molecules which mix uniformly with the water molecules. This gives a mixture which is homogeneous and there is no settling. We must note here that liquid solutions also have variable compositions. Alcohol-water solutions have different strengths. Sugar-water solutions can be very sweet and not so sweet depending on the amount of sugar added.

Solid solutions are solids in which the molecules of one component are randomly mixed with the molecules of another component. An example of a solid solution is brass, an alloy of zinc and copper. Sterling silver is a solution of copper and silver.

We can now list the characteristics which are common to all solutions:

1. Each component is broken down into molecules or atoms.
2. The molecules or atoms of each component are mixed uniformly.
3. No one component will settle out.
4. Solutions are clear and transparent.
5. The composition of a solution can vary.

To complete our study of the nature of solutions we must note two properties of solutions. These properties apply to all solutions but in varying degrees. The first is the effect of mixing two substances on the total volume of the solution. When one liter of alcohol and one liter of water are mixed, the total volume is less than two liters. When sugar is dissolved in water, the volume of solution is larger than the original volume of water. Thus mixing two substances to make a solution may cause the total volume of solution to be greater or less than the total volume of liquid(s) used.

The second property is a temperature change caused by mixing two different substances. When sulfuric acid, H_2SO_4, or sodium hydroxide NaOH are dissolved in water, the solution initially becomes hot enough to boil or at least form steam. Making solutions of either H_2SO_4 or NaOH should be done slowly and carefully. Use about half the water required and add the acid or base to the water slowly. Allow time for this mixture to cool. Then add the remaining water required slowly. Most acids and bases will cause a temperature increase when mixed with water. The temperature increase results from the reaction of the water with the acid or base. Acids react with water to produce electrically charged hydrogen atoms called hydrogen ions, H^+. Bases react with water producing hydroxide ions, OH^-. These two different reactions both produce heat.

The temperature sometimes decreases when making a solution. When sodium thiosulfate, ($Na_2S_2O_3.5H_2O$), is added to water, the solution is initially cold. When you discover this problem in making a solution you must first dissolve the chemical in about half the required water. Allow time for warming. Then add the remaining required water.

Before we go to a new topic, three new terms must be introduced:

a. Solute

b. Solvent

c. Solubility

The solute is the substance which dissolves. The solvent is the substance which does the dissolving. For a solution involving a solid mixed with a liquid, the solid is considered the solute and the liquid is the solvent. When a liquid is mixed with water, the water is the solvent and the other liquid is the solute.

Examples

1. A salt-water solution	Solute-salt Solvent-water
2. An alcohol-water solution	Solute-alcohol Solvent-water

3. An acid-water solution — Solute-acid
Solvent-water

Solubility is a term which describes the maximum amount of solute which will dissolve in the solvent. Solubility is a property of the solute not the solvent. Table salt, (NaCl), will dissolve in water. The maximum amount is 31.1 g. in every 100 g. of water. If the solvent is alcohol only 0.051 g. of NaCl will dissolve in 100 g. of solvent. When gasoline is the solvent the solubility of sodium chloride is 0.000 g. per 100 g. of solvent. You can see that the solubility of a solute will change when the solvent is changed.

The solubility of a solute in a specific solvent can be affected by temperature changes. In general, the solubility of solids increases with an increase in the temperature of the solvent. The solubility of sodium nitrate, $NaNO_3$, in water is 75 g. per 100 g. of water at 0° C. and 127 g. per 100 g. of water at 60° C.

The solubility of gases decreases with an increase in the temperature of the solvent. The solubility of oxygen in water is about 15 mg. in 1 liter of water at 0° C. and about 9 mg. in 1 liter of water at 20° C.

The subject of solubility and the variable nature of the solubility of a solute suggests the problems of specifying the actual amount of solute dissolved in a solvent. The problem is particularly important since many chemicals must be dissolved in water before they can be used. To solve the problem chemists have developed a number called the "concentration" of the solution. The concentration number describes the amount of solute in a convenient volume of solution. Suppose 1 liter of solution contains 100 g. of potassium iodide. The concentration is 100 g. per liter of solution or simple 100 g/l of potassium iodide in water. If five liters of solution contains 750 g. of salt then the concentration is 750 g. per 5 liters. Since 5 liters is not a "convenient" volume, we use a proportion to find that the concentration is 15 g/l even though there are actually 5 liters of solution.

The concentration of a solution can be found directly using the formula below:

$$\text{Concentration} = \frac{\text{weight of solute}}{\text{volume of solution}}$$

For example, 600 mg. of NaCl is dissolved in 0.5 l of solution. The concentration is:

$$\text{Concentration} = \frac{600 \text{ mg.}}{0.5 \text{ l}}$$

Now we simplify the concentration number by dividing the denominator and the numerator by 0.5

$$\text{Concentration} = \frac{1200 \text{ mg.}}{1 \text{ liter}} \qquad \text{or } 1200 \text{ mg/liter}$$

The concentration is normally reported in the units mg/l, g/l or ppm. If the weight and volume data are given in units other than milligrams or grams and liters, you can change the given units by the appropriate conversion factors. Then use the formula given. Remember that

1 mg/l = 1 ppm

1000 mg/l = 1 g/l

Two other units of concentration commonly used in chemistry are normality (N) and molarity (M). These are examples of the two units:

0.25N H_2SO_4 -- means a .025 normal solution of sulfuric acid.

2 M NaOH -- means a 2 molar solution of sodium hydroxide.

Module No:	**Module Title:** Basic Laboratory Skills
	Submodule Title: General Skills
Approx. Time: 1 hour	**Topic:** Dilution Techniques

Objectives:

Upon completion of this module, the participant should be able to:

1. Make single step and multiple step dilutions of concentrated samples and perform calculations taking into account dilution factors.

Instructional Aids:

Handout: "Dilution Techniques"
Pipets
Dilution Blanks

Instructional Approach:

Lecture
Demonstration
Laboratory Practice

References:

Standard Methods for the Examination of Water and Wastewater, 14th Edition

Class Assignments:

Read handout
Participate in laboratory practice sessions

Module No:	Topic: Dilution Techniques

Instructor Notes:	Instructor Outline:
Handout: "Dilution Techniques"	Discuss dilution techniques. Demonstrate dilution techniques. Discuss and demonstrate calculations related to dilutions.

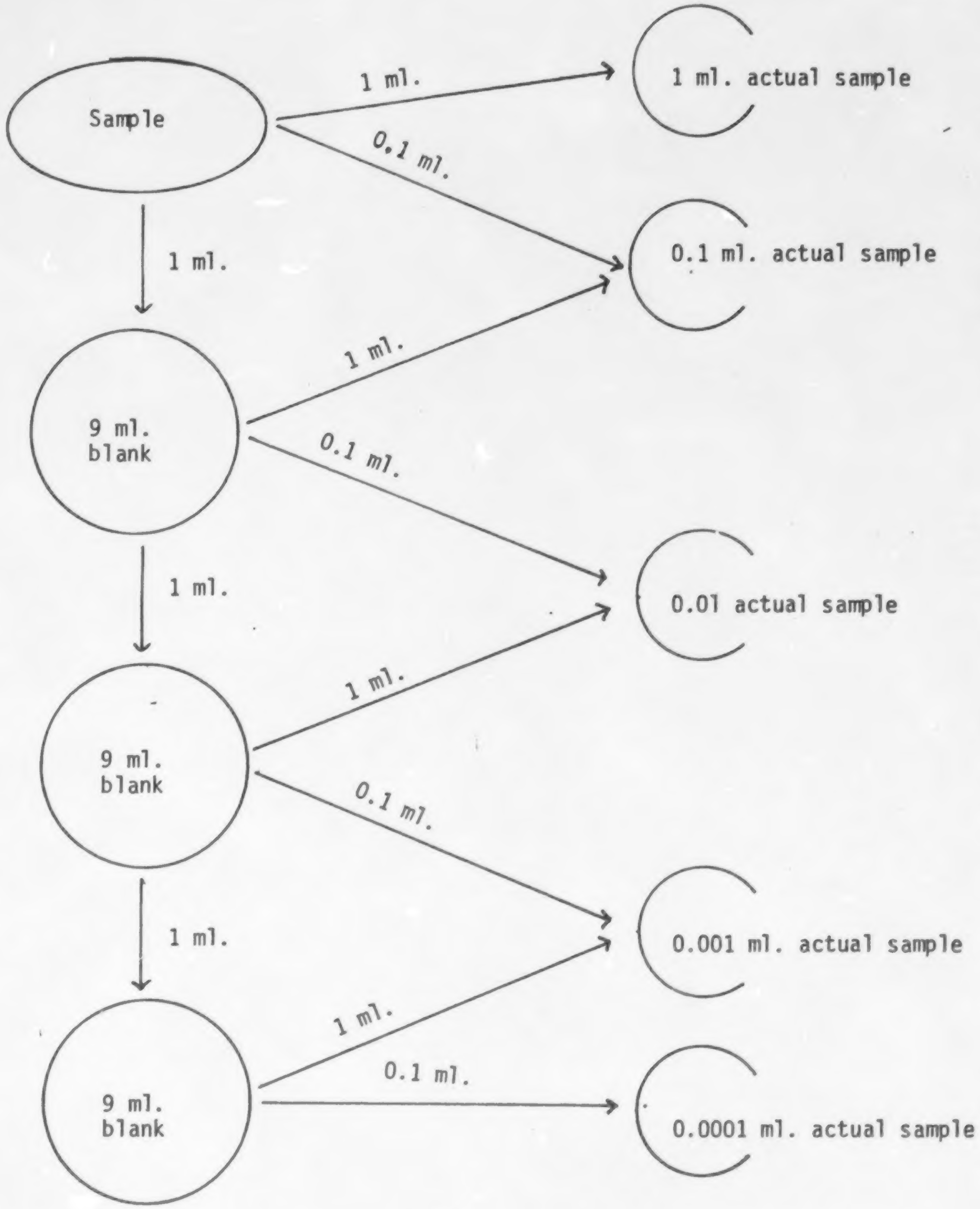
DILUTION TECHNIQUES

Sample dilution is necessary when the concentration of the entity being measured is too great to be determined by the technique employed. By diluting the sample with distilled water, or other solution free of the entity being measured, its concentration can be brought within the range where it can be accurately measured.

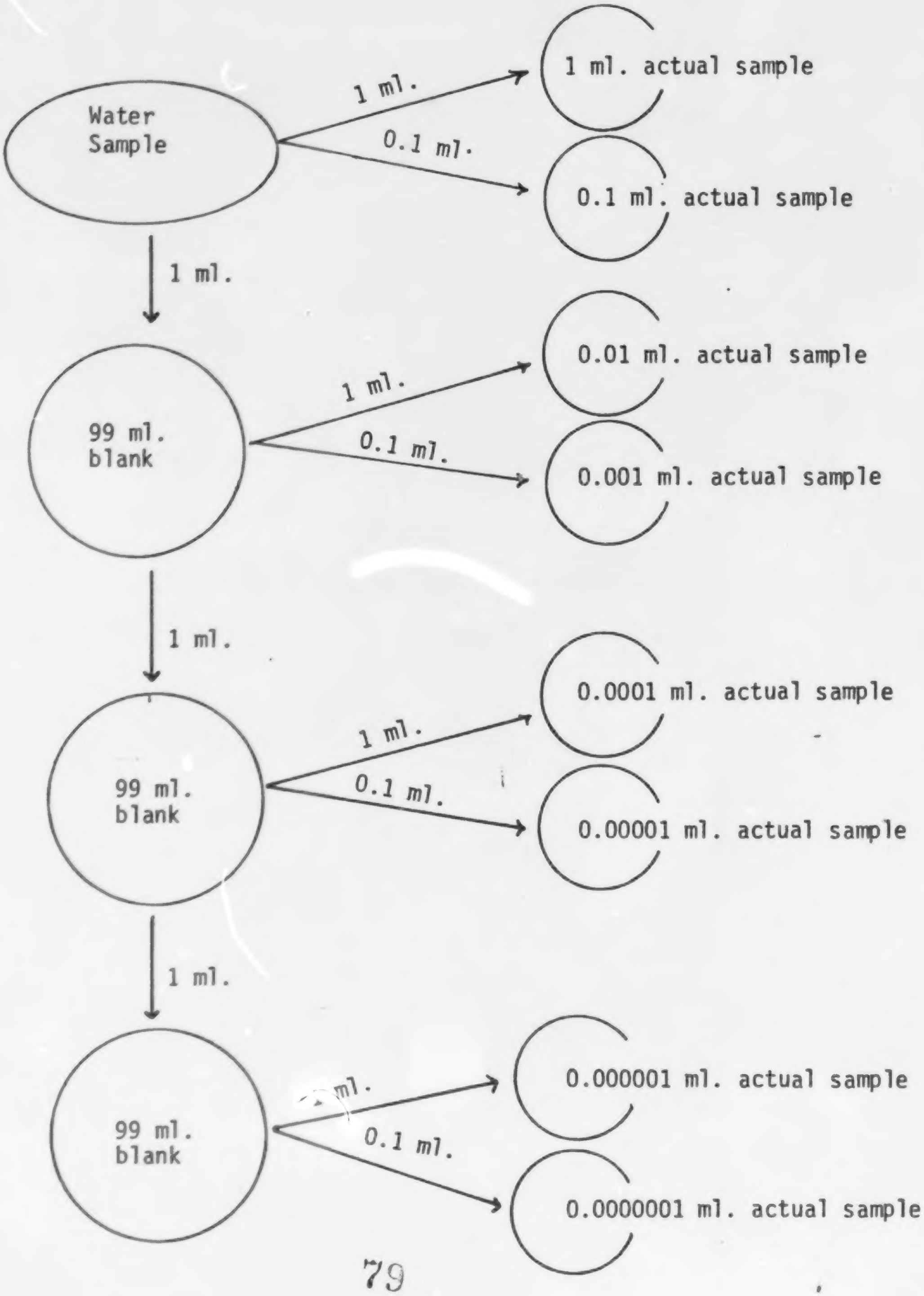
There are two basic methods of dilution, serial dilution and parallel dilution. In serial dilution a known volume is transferred to a dilution blank plus the sample is used for the next transfer 1:10 and 1:100 serial dilutions are shown on pages 75 and 76.

Parallel dilutions are made by always removing a known volume from the sample bottle and using dilution blanks of various sizes to make the proper dilutions. This technique is diagrammed on page 77.

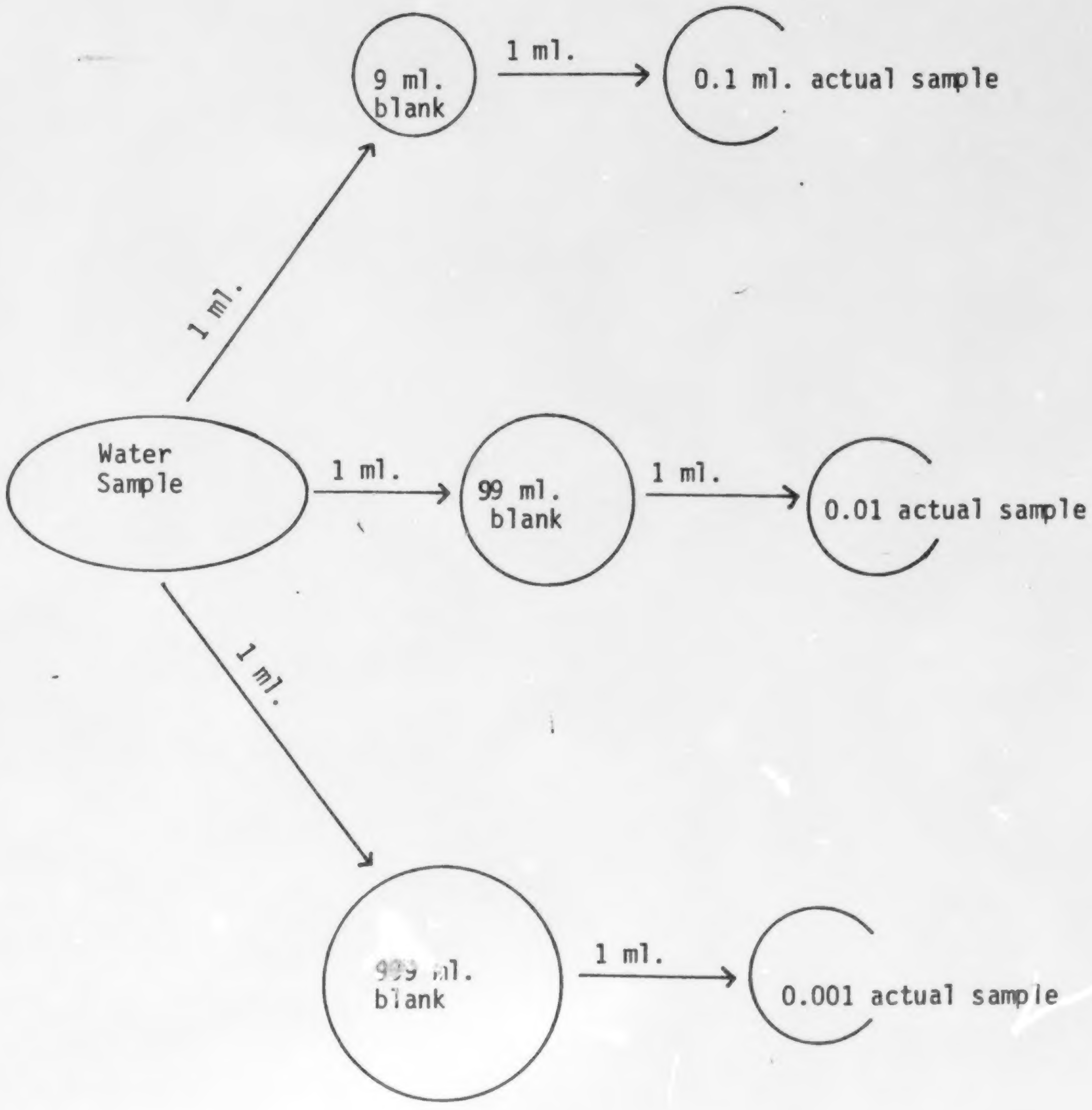
1:10 Serial Dilution Technique


Sample
1 ml.
0.1 ml.
1 ml. actual sample
0.1 ml. actual sample
1 ml.
9 ml. blank
1 ml.
0.1 ml.
0.01 actual sample
1 ml.
9 ml. blank
1 ml.
0.1 ml.
0.001 ml. actual sample
1 ml.
9 ml. blank
1 ml.
0.1 ml.
0.0001 ml. actual sample

1:100 Serial Dilution Technique


Water Sample
1 ml.
0.1 ml.
1 ml. actual sample
0.1 ml. actual sample
1 ml.
99 ml. blank
1 ml.
0.1 ml.
0.01 ml. actual sample
0.001 ml. actual sample
1 ml.
99 ml. blank
1 ml.
0.1 ml.
0.0001 ml. actual sample
0.00001 ml. actual sample
1 ml.
99 ml. blank
ml.
0.1 ml.
0.000001 ml. actual sample
0.0000001 ml. actual sample

Parallel Dilution Techniques


Water Sample
1 ml.
9 ml. blank
1 ml.
0.1 ml. actual sample
1 ml.
99 ml. blank
1 ml.
0.01 actual sample
1 ml.
999 ml. blank
1 ml.
0.001 actual sample

Module No:

Module Title:

Basic Laboratory Skills

Submodule Title:

General Skills

Approx. Time:

1/3 hour

Topic:

Incubators

Objectives:

Upon completion of this module, the participant should be able to:

1. State precautions applicable to the care and use of all incubators.

Instructional Aids:

Handout

Instructional Approach:

Lecture
Discussion

References:

1. Standard Methods for the Examination of Water and Wastewater, 14th Edition.
2. Carnegie and Wooley, Laboratory Manual for Sanitary Microbiology and Sanitary Chemistry, EPA, 1975.

Class Assignments:

Read handout

Module No:	Topic: Incubators
Instructor Notes:	**Instructor Outline:**
1. Include discussions of: a. Installation b. Temperature sensitivity c. Humidity d. Loading e. Cleaning f. Differences	1. Discuss the precautions which must be taken when using gravity convection and forced air inoculators.
2. Include discussion of: a. Installation b. Temperature sensitivity c. Loading d. Cleaning e. Differences	2. Discuss the precautions which must be taken when using convection and circulated water bath incubators.

Module No:	**Module Title:** Basic Laboratory Skills
	Submodule Title: General Skills
Approx. Time: 2 hours	**Topic:** Balances

Objectives:

Upon completion of this module, the participant should be able to:

1. State precautions applicable to the care and use of all balances.
2. Identify and use a triple beam balance with a range of 0 - 100 g. with an accuracy of ± 0.01 g. given the balance and appropriate reference material.
3. Identify and use an analytical balance with an accuracy of ± 0.0002 g. given the balance and appropriate reference material.

Instructional Aids:

Analytical Balance
Weights
Beam Balance

Instructional Approach:

Demonstration
Lab

References:

Analytical Quality Control, USEPA, Technology Transfer

Class Assignments:

Participate in laboratory practice sessions

Module No:

Topic:
Balances

Instructor Notes:

Instructor Outline:

1. Discuss care and preventive maintenance of balance.
2. a. Discuss and demonstrate the use of a triple beam balance.
   b. Have participants use a triple beam balance.
3. a. Discuss and demonstrate the use of an analytical balance.
   b. Have participant weigh an object on an analytical balance.
   c. Have participant weigh an object on two different analytical balances. Compare the weights. Discuss the consequences of the results.
4. Discuss the use of other types of balances. Ex. electronic

Module No:

Module Title:

Basic Laboratory Skills

Submodule Title:

Chemistry Skills

Approx. Time:

1 hour

Topic:

Analytical Analysis

Objectives:

Upon completion of this module, the participant should be able to:

1. Differentiate between volumetric gravimetric and colorimetric analysis.
2. Differentiate between precision and accuracy.

Instructional Aids:

Volumetric glassware, filtration setup
Spec. 20 of filter photo meter
Overheads
1. Precision and accuracy
Handouts
1. Laboratory analysis
2. Precision and accuracy

Instructional Approach:

Lecture
Demonstration

References:

Simplified lab procedure for Wastewater Examination, WPCF, 1971.
Analytical Quality Control, USEPA TEchnology Transfer

Class Assignments:

Read handout

Module No:

Topic: Analytical Analysis

Instructor Notes:	Instructor Outline:
Handout: Laboratory Analysis	1. Discuss volumetric analysis, gravimetric analysis, colorimetric analysis.
	2. Demonstrate an example of each type of analysis.
Show using a known volume and concentration to determine the concentration of second known volume.	
Show filtration and weighing.	
Show the development of a color in proportion to concentration.	
Overheads Presicision and Accuracy	3. Define Precision and Accuracy
Handout: Precision and Accuracy	Discuss precision and accuracy and how they relate to average and standard deviation.

LABORATORY ANALYSIS

The laboratory analysis of wastewater deals with the detection and quantitative estimation of the substances present in wastewater and the effects of these substances on the treatment process. In one type of analysis known as "qualitative analysis", the operator sets out to detect the different substances that may be present in the wastewater being tested. In "quantitative analysis", the operator attempts to determine exact amounts, by weight or by volume, of the various substances in a known weight or volume of the wastewater sample. Quantitative analyses are made volumetrically, gravimetrically, or colorimetrically.

Volumetric Analysis

In laboratory procedures classified as volumetric analyses, the operator measures the amount of a solution of known concentration that reacts quantitatively with a particular substance in the solution of a weighed or otherwise measured portion of the original sample. The weight of the material being sought is found indirectly from the amount of the known (standard) solution that is required. The means of detecting the completion or "end-point" of the volumetric reaction is the indicator. The process of finding the amount of the standard solution required is called a "titration".

Gravimetric Analysis

In laboratory procedures classified as gravimetric analyses, the operator measures the sample of wastewater or sludge and then isolates and weighs an element or one of its compounds. Examples of the gravimetric type of analyses are total solids (residue on evaporation) and volatile solids and suspended solids.

Colorimetric Analysis

Colorimetric methods of analyses have been developed for several determinations in an effort to find faster, more economical, and convenient ways of obtaining quantitative laboratory data. For a colorimetric method to be quantitative, it must form a compound with definite color characteristics which are directly proportional to the concentration of the substance being measured. Colorimetric measurements may be made in a wide range of equipment. The wastewater treatment plant operator may use standard color-comparison tubes, photoelectric colorimeters, or spectrophotometers. Each has its place and particular application in wastewater analysis. Color comparison tubes, sometimes referred to as Nessler tubes, have been standard equipment for making colorimetric measurements for many years. Precise work with color comparison tubes requires the use of tubes of matched size. The main difficulty with their use is that the standard color solutions often are unstable and every time a determination has to be made it becomes necessary to prepare a series of fresh standards. The use of color tubes and standards is being replaced rapidly by the photoelectric and spectrophotometric methods largely because of convenience and accuracy.

ACCURACY AND PRECISION

Accuracy is defined as the closeness of a measurement or series of similar measurements to the true value of the quantity measured.

In contrast, precision or repeatability might be defined as the closeness of a number of measurements to a common value, but not necessarily the true value. Precision is desirable but its attainment is not proof that an accurate series of measurements has been made, since constant sources of error may enter into all of the measurements in a series. These errors might fall into one of two classes, some being determinate and others indeterminate. The determinate errors may be discovered, and corrected for or eliminated; while the indeterminate errors essentially are obscured and unknown.

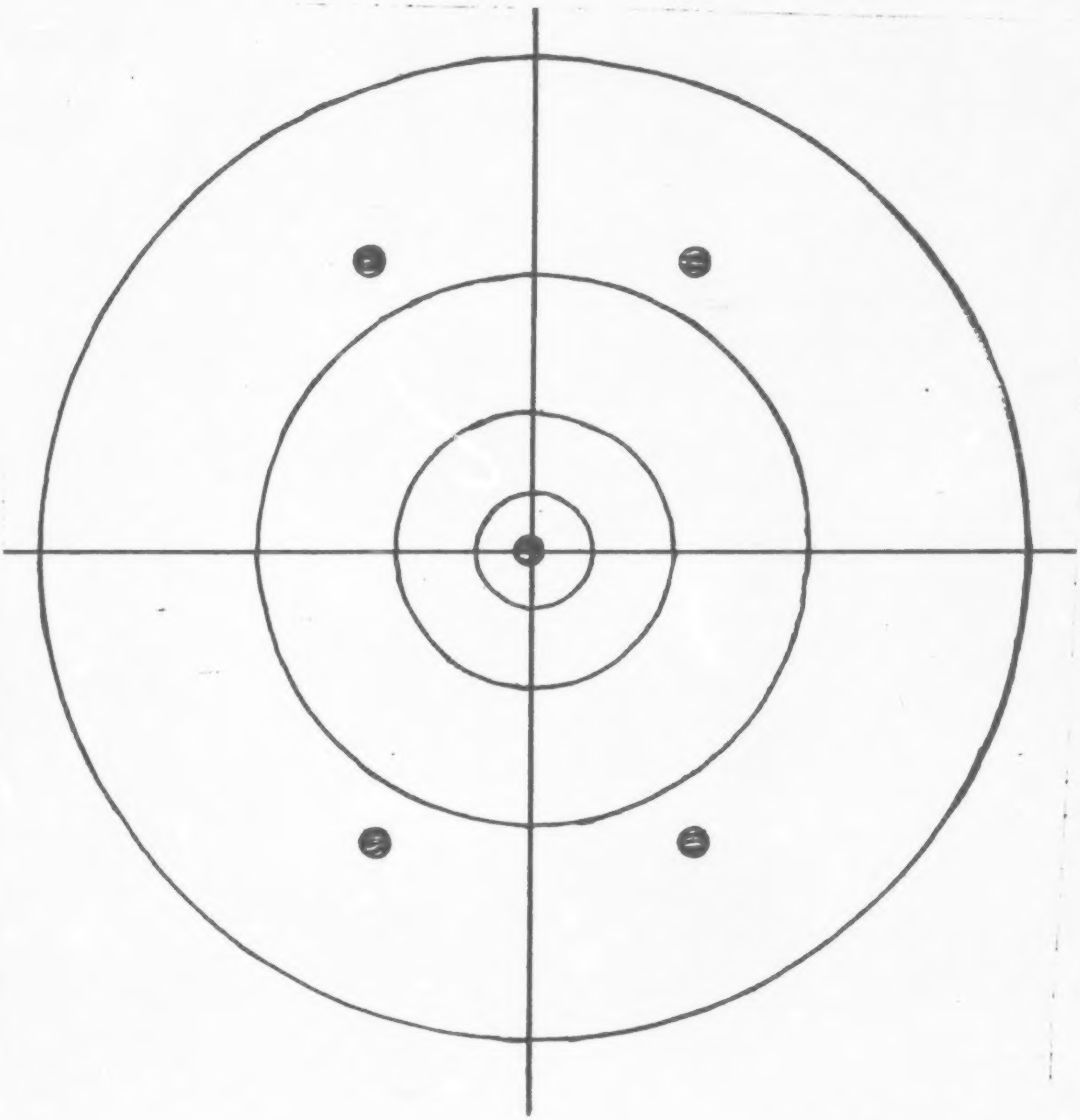
Determinate errors may be:

1. Personal errors due to factors for which the operator is responsible, such as neglecting to read a buret properly, inability to identify color changes, failure to mix volumetric solutions completely, or mis-reading values marked on small weights.
2. Instrumental errors due to the instruments. Imperfect weights, volumetric glassware, and balances are sources of instrumental error.
3. Errors in method, including those due to such things as the use of an improper temperature or time of drying of a solids sample.

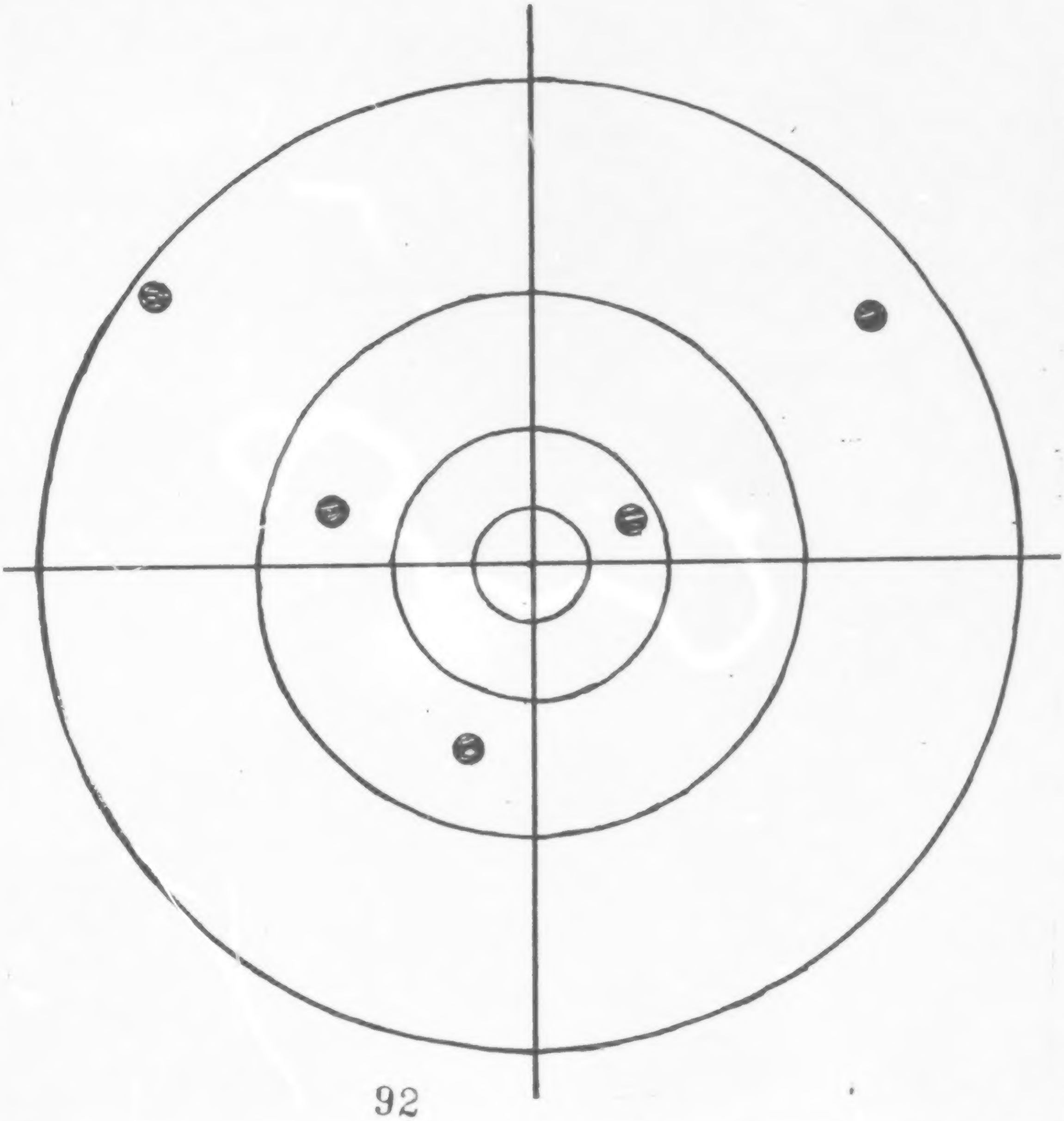
In general, no laboratory result should be rejected except for an obvious source of error. Measurements that vary widely from the mean (or average) may be omitted when determining an average if a reasonable explanation is given. For instance, in a series of four parallel observations or determinations, if one of the four is greatly different from the other three, it might be omitted.

In any measurement only one uncertain figure should be retained. An uncertain figure is the result of an estimate between division on a scale. For example, on a buret which is calibrated only to tenths of ml, the reading would be estimated to the nearest hundredth. Weights in grams should be recorded with four figures to the right of the decimal point (for example, 4.3267 g). Following the rule that only one uncertain figure is retained in recording a measurement, the numbers thus set down are considered to be significant figures. In rounding off measured or computed quantities to the proper number of significant figures 1 should be added to the last significant figure in the next position is 5 or greater. For example, in weighing 4.32567 g would be rounded off to 4.3257 g.

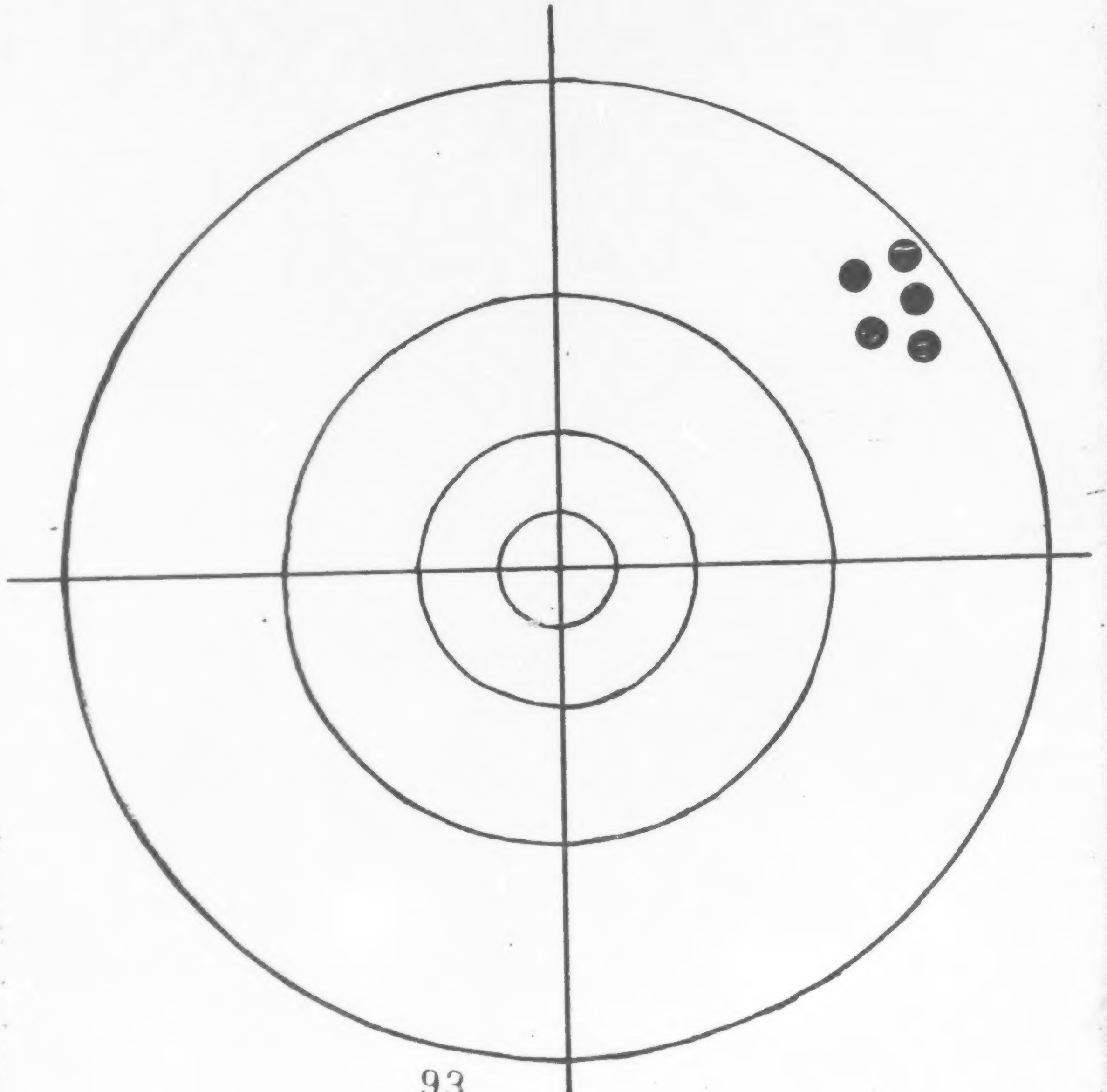
HIGH ACCURACY LOW PRECISION.



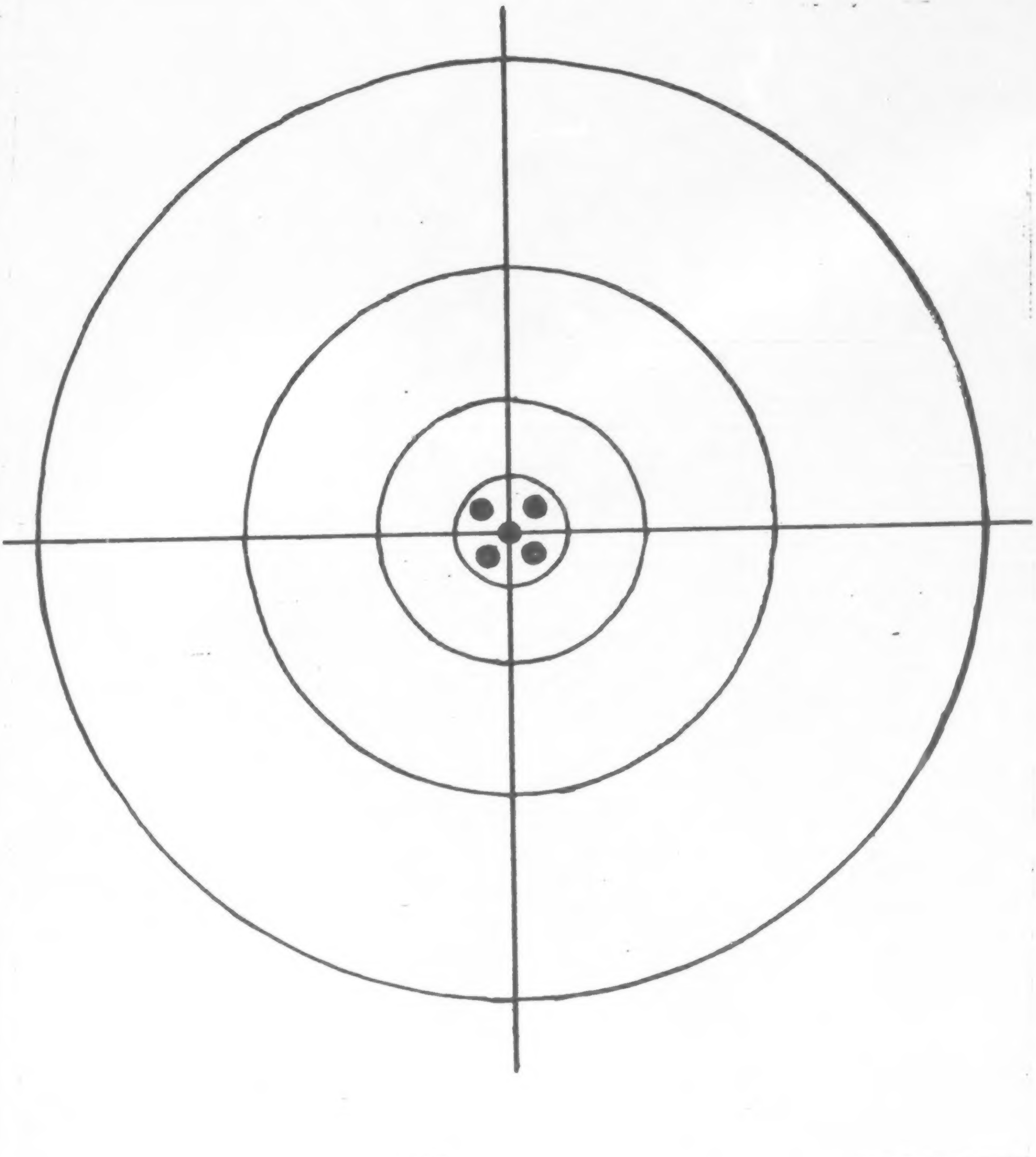
LOW ACCURACY LOW PRECISION



LOW ACCURACY HIGH PRECISION



HIGH ACCURACY HIGH PRECISION



Module No:

Module Title:
Basic Laboratory Skills

Submodule Title:
Chemistry Skills

Approx. Time:
3 hours

Topic:
Volumetric Glassware

Objectives:

Upon completion of this module, the participant should be able to:

1. Demonstrate the proper use of the following types of volumetric glassware: Buret, volumetric flask, pipet.
2. Indicate the difference between glassware calibrated to contain and to deliver.
3. Conduct a titration of a strong acid with a strong base using a color end point using proper volumetric technique.

Instructional Aids:

Handout: Volumetric Glassware
Volumetric glassware
Titration setup

Instructional Approach:

Lecture
Demonstration
Lab

References:

Standard Methods, 14th Edition
Analytical Quality Control, USEPA, Technology Transfer

Class Assignments:

Read Handoug
Participate in laboratory practice session

Module No:	Topic: Volumetric Glassware

Instructor Notes:	Instructor Outline:
Titration Dilute 10 ml. of 1 N H_2SO_4 to 100 ml. and titrate with 0.1 N NaOH.	1. a. Demonstrate the proper use of a durey volumetric flask and pipet. b. Conducting a titration.
	2. Discuss use of glassware calibrated to contain and to deliver. Indicate when each is to be used.
(S.P. Duopette)	Show a pipet calibrated to contain and to deliver.
1 N H_2SO_4 acid .1 N NaOH Base Phenolphythalein end point.	3. Have participant practice titration technique by diluting an acid and titrating it with a base using a color endpoint.
Handout: Volumetric Glassware	

BURETTES

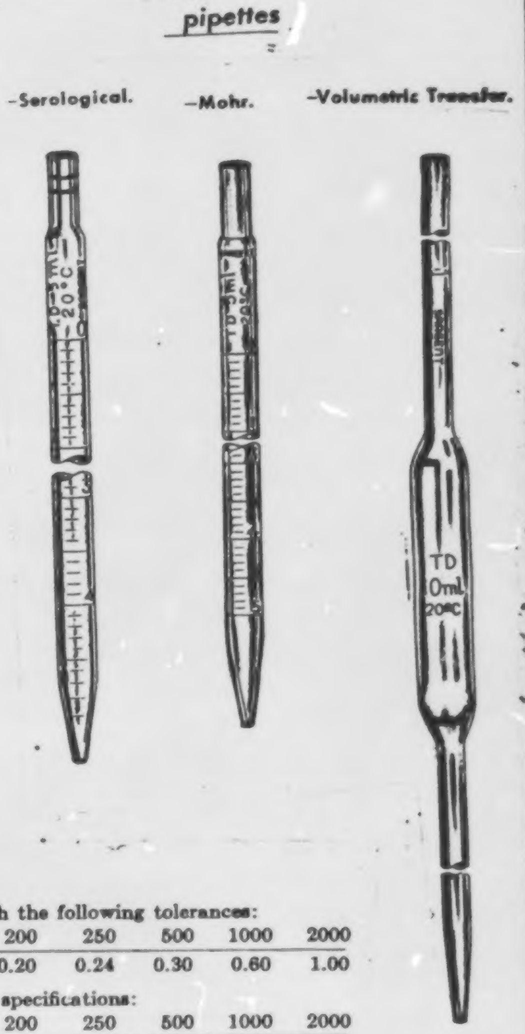
Burette Accuracy Tolerances

Delivery, ml	10	25
Class A (Precision Grade)	±0.02	±0.03
Other than Class A	±0.04	±0.06

Delivery, ml	50	100
Class A (Precision Grade)	±0.05	±0.10
Other than Class A	±0.10	±0.20

Automatic burettes with overflow orifice for filling are very convenient for rapid repetitive titrations but cannot be relied upon to deliver within the accuracy tolerances shown in the table above because of the somewhat inconsistent establishment of initial level at the overflow aperture.

Schellbach burettes, which are considered very easy to read, cannot be guaranteed to fall strictly within the stated tolerances because of personal variables in reading this type of burette.



Volumetric Flasks

Flasks not described as Class A are calibrated at 20°C with the following tolerances:

Capacity, ml	10	25	50	100	200	250	500	1000	2000
Tolerance, ± ml	0.06	0.06	0.10	0.16	0.20	0.24	0.30	0.60	1.00

Flasks designated "Class A" are calibrated to meet N.B.S. specifications:

Capacity, ml	10	25	50	100	200	250	500	1000	2000
Tolerance, ± ml	0.03	0.03	0.05	0.08	0.10	0.12	0.15	0.30	0.50

PIPETTES

Tolerances For Analytical Grade Pipettes

Transfer Pipettes				*Measuring and Serological Pipettes*	
Size	*Tolerance*	*Size*	*Tolerance*	*Size*	*Tolerance*
1 ml	±0.012 ml	15 ml	±0.06 ml	0.1 ml	±0.005 ml
2 ml	±0.012 ml	20 ml	±0.06 ml	0.2 ml	±0.008 ml
3 ml	±0.02 ml	25 ml	±0.06 ml	1 ml	±0.02 ml
4 ml	±0.02 ml	50 ml	±0.10 ml	2 ml	±0.02 ml
5 ml	±0.02 ml	100 ml	±0.16 ml	5 ml	±0.04 ml
10 ml	±0.04 ml	200 ml	±0.20 ml	10 ml	±0.06 ml
				25 ml	±0.10 ml
				50 ml	±0.16 ml

Module No:

Module Title:
Basic Laboratory Skills

Submodule Title:
Chemistry Skills

Approx. Time:
3 Hours

Topic:
Standardization of Reagents

Objectives:

Upon completion of this module, the participant should be able to:

1. Indicate the relationship between normality and volume of two neutralizing solutions at the equivalence point.
2. Calculate the weight of solute needed to make a solution of stated normality given a list of equivalent weights.
3. Standardize a solution given a neutralizing primary standard, balance and volumetric glassware.

Instructional Aids:

Titration setup

Instructional Approach:

Lab

References:

Standard Methods, 14th Edition

Class Assignments:

Module No:	Topic: Volumetric Analysis

Instructor Notes:	Instructor Outline:
	1. a. Explain the equation ml x N = ml x N b. Work examples using data from topic on volumetric glassware.
	2. a. Discuss how the normality of a solution can be determined. Give the equivalent weight of the solute, weight of solute and volume of solution. b. Work examples
KHP EWT = Base .1 N	3. a. Define primary standard b. Have participant weigh out a given amount of primary standard and titrate it with a base. c. Have participant calculate the exact normality of the base from the above data.

Module No:	**Module Title:** Basic Laboratory Skills
	Submodule Title: Chemistry Skills
Approx. Time: 3 hours	**Topic:** Colorimetric Analysis

Objectives:

Upon completion of this module, the participant should be able to:

1. Indicate the relationship between concentration and absorbance.
2. Identify the basic components of a Spec 20 or other common laboratory spectrophotometer.
3. Set up, standardize and use a Spec 20 to determine the absorbance of a sample, given a Spec 20, sample, operating instructions and wave length of max absorbance of the sample.
4. Indicate the relationship between absorbance and transmittance.

Instructional Aids:

EPA video tape
Overheads
Handout: "Colorimetric Analysis"

Instructional Approach:

Lecture
Lab

References:

Effluent Monitoring Procedures, Nutrients, USEPA

Class Assignments:

Read handout
Participate in laboratory practice sessions

Module No:	Topic: Colorimetric Analysis

Instructor Notes:	Instructor Outline:
	1. a. Discuss Beer's Law
	b. Demonstrate Beer's Law using nessler tubes and a $KMnO_4$ solution.
Spec 20 Overheads	2. a. Identify the components of a Spec 20.
Handout: Colorimetric Analysis	b. Explain how each component works and what its purpose is in the Spec 20.
EPA video tape	3. a. Demonstrate the use of a Spec 20.
	b. Have participants set up, standardize and use the Spec 20.
	4. Discuss the relationship between absorbance and transmittance.

COLORIMETRIC ANALYSIS

Use of a Spectrophotometer

General Description of Equipment Used in the Process

A. Capital

1. One Bausch and Lomb Spectronic 20 Spectrophotometer
2. One manufacturer's manual for the spectrophotometer
3. Still, or other source of distilled water
4. Hotplate
5. One spectrophotometer cell - A set of cells may be used only if the cells are optically matched. One cell would be used for each solution.

B. Reusable

1. Brush (for cleaning spectrophotometer cell)
2. Laboratory apron
3. Safety glasses
4. One pen or pencil
5. Notebook or data sheet (see page 1-23) for recording data
6. Brush (for dusting spectrophotometer)
7. One 2 liter beaker
8. One 250 ml. beaker
9. One glass stirring rod
10. One 2 liter glass stoppered bottle
11. One visible phototube (Bausch and Lomb catalog number 33-29-71)
12. One infrared phototube (Bausch and Lomb catalog number 33-29-72)
13. One infrared filter (Bausch and Lomb catalog number 33-29-18)

14. Ten soft tissues (for wiping the cells)
15. One plastic squeeze distilled water bottle
16. Sink or 1 liter container for rinsing solutions
17. One 1 cm. cell (to fit the Spectronic 20)

C. Consumable

1. Soap
2. Sodium dichromate, $Na_2Cr_2O_7$
3. Concentrated sulfuric acid, H_2SO_4

Items A4, B7 through B10, and C1 through C3 for cleaning the spectrophotometer cell.

Use of a Spectrophotometer

1. Analysis Objectives:

   The user of the attached effluent monitoring procedure will learn how to use the Bausch and Lomb Spectronic 20 Spectrophotometer for making colorimetric measurements.

2. Brief Description of Analysis:

   In the field of water pollution analysis, many determinations are based on measuring the intensity of color at a particular wavelength. In general, color is formed in the sample by some sort of preliminary treatment such as distillation or digestion, and then adding a color developing reagent. The intensity of the color formed is related to the amount of material (such as phosphorus) in the sample. As part of the analysis, color is also developed in a series of standards; in each of the standards is a known amount of the material (such as phosphorus) of interest. A calibration curve is made using the color intensities of the individual standards and the corresponding amounts of material present. The amount of material present in the sample is determined using the calibration curve. A Bausch and Lomb Spectronic 20 Spectrophotometer is an instrument used to measure the color intensities of the standards and sample. The word absorbance is associated with the words color intensity; i.e. a sample or standard which has a low color intensity will also have a low absorbance.

A. Equipment Preparation

1. Cell cleaning

Clean the Bausch & Lomb Spectronic 20 Spectrophotometer test tube cell.

a. For the rest of this effluent monitoring procedure the abbreviation "Spec 20" will be used.

2. Spec 20 cleaning

Clean the Spec 20.

a. It should be free of dust, dirt, and spilled chemicals.

b. The Spec 20 should be stored in an area where there is no danger that chemicals will be spilled on it.

c. The plastic cover supplied with the Spec 20 should be covering the instrument whenever it is not in use.

If the power cord is plugged into a wall outlet, remove it.

3. Phototube

Check whether the proper phototube is in place.

a. See Section C for instructions on changing the phototube and inserting the filter.

b. On the wavelength scale, note that below about 625 nm, the numbers are in black, and that above 625 nm, the numbers are in red.

c. If the wavelength to be used in the particular phototube (Bausch & Lomb Catalog number (33-29-71) should be used.

d. If the wavelength to be used is in the red zone, the infra-red phototube (Bausch & Lomb Catalog number 33-29-72) and infra-red filter (Bausch & Lomb Catalog number 33-29-18) should be used.

B. Spec 20

1. Warm up

   Plug the power cord into a wall outlet

   a. 115 V, A.C., 60 Hz

2. Turn the power switch/zero control knob (see figure 1) clockwise, until a click is heard.

   a. The instrument is now turned on.

   b. If there is a pilot light on the instrument. it will also be on.

   c. The sound of the cooling fan may also be heard.

3. Turn the power switch/zero control knob an additional one half clockwise turn.

   a. This will keep the needle from "pegging" during the warm up period.

4. Wait ten minutes

   a. This is the warm up period.

   b. Ten minutes are generally specified in the manufacturer's manual. However, longer warm up periods than those specified generally give better instrument stability.

   c. If the Spec 20 is old, a longer than 10 minute warm up period may be required. Twenty to thirty minutes would be a suitable warm up time.

Operation

1. Assemble the standards and samples whose color intensities are to be measured.

2. Set the wavelength control to the desired setting.

   a. This setting will be specified in the procedure you are using to determine the particular parameter.
   b. Always approach the desired setting by turning the knob clockwise.
3. If the sample holder cover is open, close it.
   a. It should be closed unless a cell is being inserted or removed.
4. Turn the power switch/zero control knob until the needle reads infinite (symbol) absorbance.
   a. Use the absorbance (lower) part of the scale. The other (upper) half of the scale is marked in transmittance.
   b. The words absorbance and color intensity are related; i.e. if a solution has a low color intensity, it will also have a low absorbance.
5. Fill the cell with the blank.
   a. Also sometimes called the zero standard.
6. Empty the cell into the sink.
7. Fill the cell with blank.
8. Empty the cell into the sink.
   a. The cell has now been rinsed twice with solution.
9. Fill the cell with blank.
   a. Three fourths full. Estimate this volume.
10. Thoroughly wipe the outside of the cell with a tissue.
    a. So as to remove finger prints and any spilled solution.
11. Open the sample holder cover.

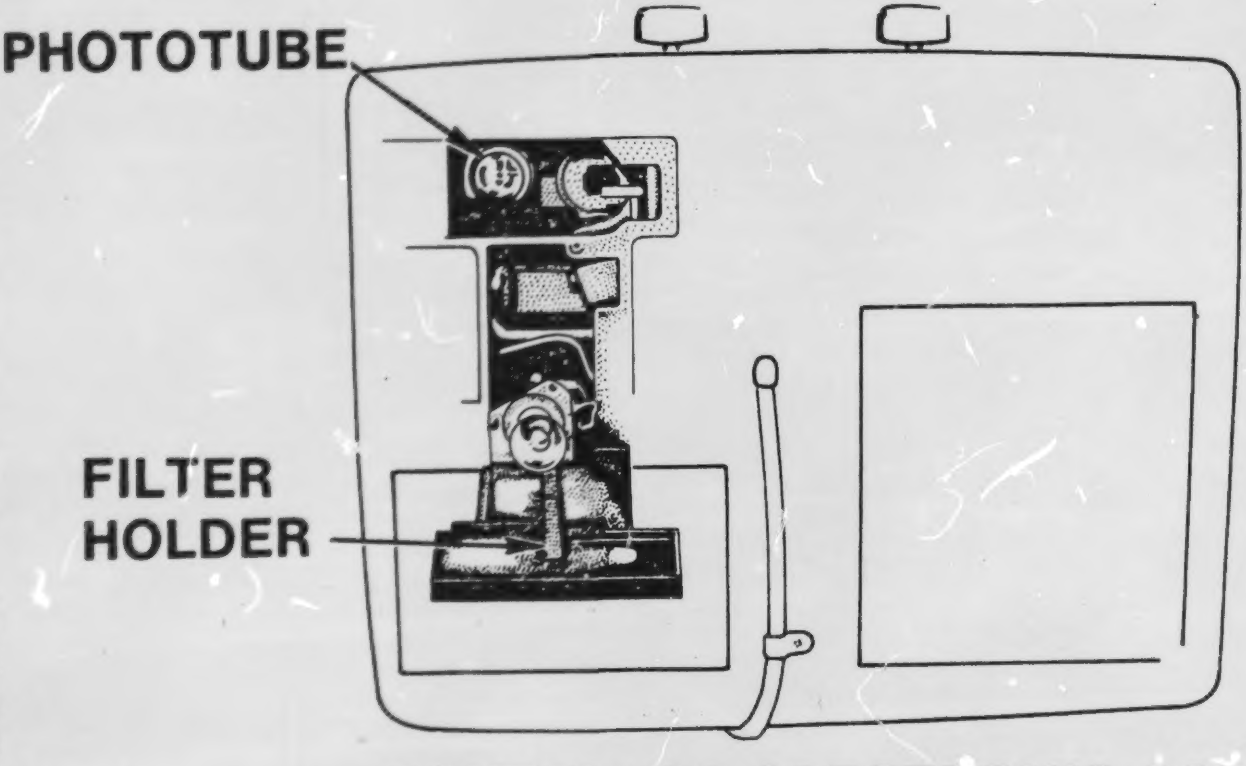
12. Slowly and gently slide the cell down into the sample holder as far as it will go.
    a. Do not force the cell down.
    b. The needle will move away from the infinite absorbance setting.
13. Slowly rotate the cell until the white vertical line on the cell is in line with the ridge on the edge of the sample holder (see figures 2 and 3).
14. Close the sample holder cover.
15. Turn the light control knob until the needle reads zero absorbance.
    a. Use the absorbance scale for all of the readings.
16. Record an absorbance of zero and a concentration of zero for this solution.
    a. An example data sheet is on page 23.
17. Raise the sample holder cover.
18. Slowly remove the cell.
    a. No solution should be spilled on the inside of instrument.
19. Close the cover.
    a. The needle should return to the infinite absorbance setting. If it does not, reset it with the power switch/zero control knob.
    b. If it was necessary to reset the infinite absorbance reading, repeat steps 11 through 15.
20. Empty the contents of the cell into the sink.
21. Fill the cell with tap water.
22. Empty it into the sink.

23. Fill the cell with tap water.
24. Empty it into the sink.
25. Fill the cell with distilled water.
26. Empty it into the sink.
27. Fill the cell with distilled water.
28. Empty it into the sink.
29. Fill the cell with the next solution whose color intensity (absorbance) is to be measured.
    a. In a set of standards, the absorbance of the lowest concentration standard is measured second, and so on, to the highest concentration standard.
30. Empty it into the sink.
31. Fill the cell with the same solution again.
32. Empty it into the sink.
33. Fill the cell three fourths full with the same solution.
34. Thoroughly wipe the outside of the cell with a tissue.
    a. So as to remove finger prints and any spilled solution.
35. Open the sample holder cover.
36. Slowly and gently slide the cell down into the sample holder as far as it will go.
    a. Do not force the cell down.
    b. The needle will move away from the infinite absorbance setting.
37. Slowly rotate the cell until the white vertical line on the cell is in line with ridge on the edge of the sample holder (see figure 2 & 3).
38. Close the sample holder cover.

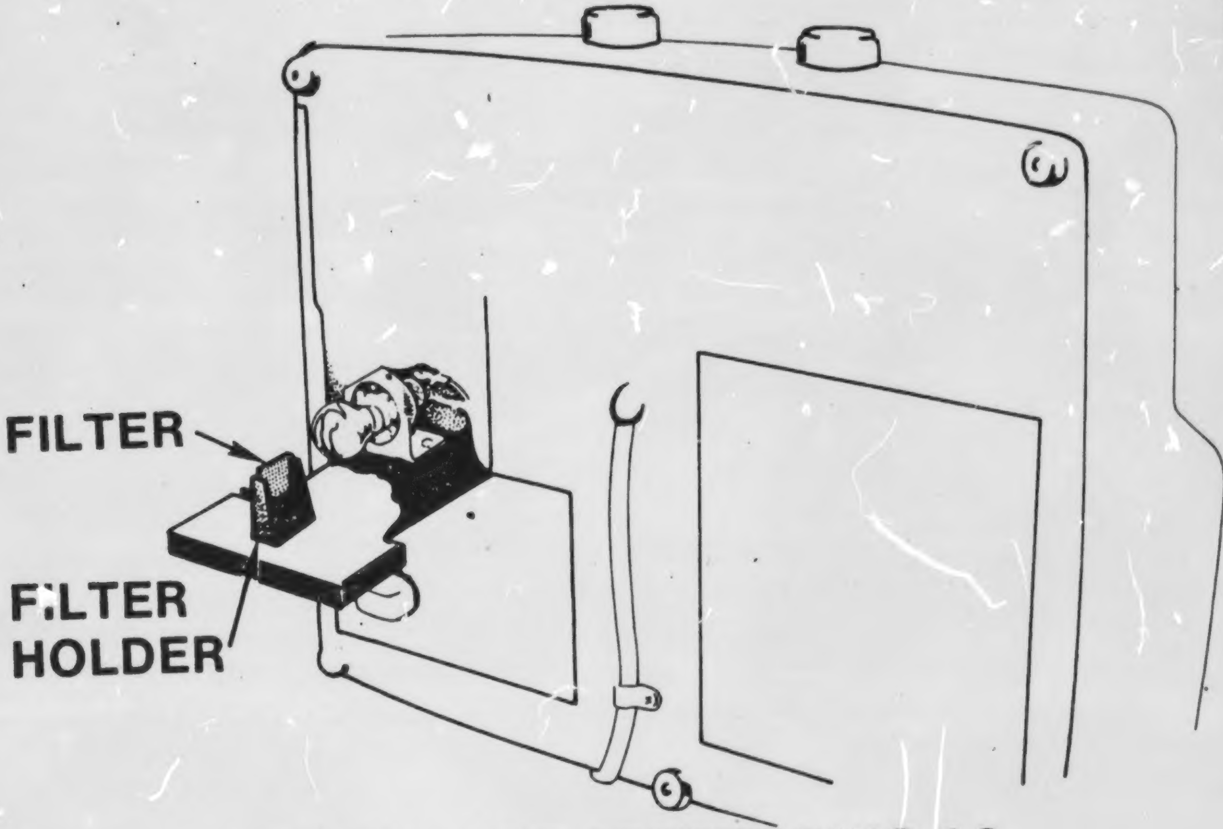
39. Record the absorbance and concentration of this solution.
    a. While looking at the absorbance scale, note that in some parts of the scale, the third place to the right of the decimal will be an estimated number, while in other parts, the second place will be an estimated number.
    b. Absorbance values of greater than 0.7 are considered to be inaccurate. For this reason, about three sample dilutions are usually done so that at least one will give an absorbance of less than 0.7. If one of the standards happens to have an absorbance of greater than 0.7, it should not be used.
    c. If a great number of measurements are to be made at a particular time (e.g., a great number of phosphorus absorbancies are to be measured), steps 4 through 15 should be repeated every fifth measurement.
    d. Recall that step 4 was done with no cell in the instrument.
    e. This is an insurance against "drifting" of the setting.
40. Using each of the rest of the standards in sequence, and samples, repeat steps 17 through 39.
41. Repeat steps 17 through 28.
42. Store the cell until it is again needed.
43. Turn the power switch/zero control knob slowly counter clockwise until a click is heard.
    a. If the instrument has a pilot light, it will go out.
    b. The Spec 20 is turned off.
44. If a plastic cover was supplied with the Spec 20, it should now be replaced.

C. Phototube Changing

1. Turn the power switch/zero control knob slowly counter-clockwise until a click is heard.
    a. The instrument may already be turned off.
    b. If the instrument has a pilot light, it will go out.
    c. The Spec 20 is turned off.
2. Remove the power cord from the wall outlet.
    a. The power cord may already be removed from the wall outlet.
3. Tilt the Spec 20 away from you.
    a. The Spec 20 should be standing on its back.
    b. The bottom of the instrument is facing you.
    c. This position is somewhat unsteady. Be careful not to knock the instrument over.
4. Steady the instrument with one hand.
5. Loosen the thumbscrew with the other hand (see figure 4).

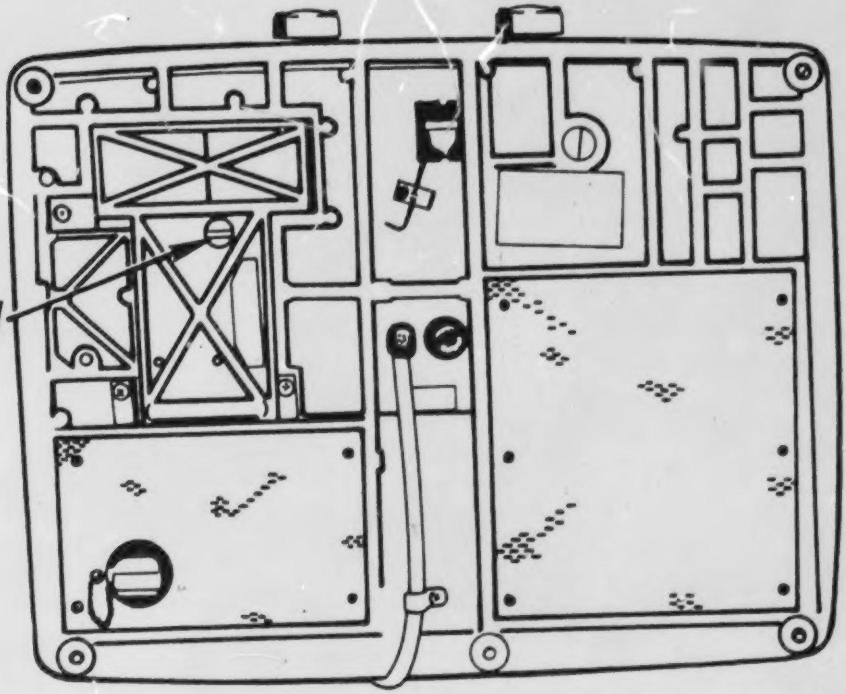

PHOTOTUBE
FILTER
HOLDER

BOTTOM OF SPECTRONIC 20

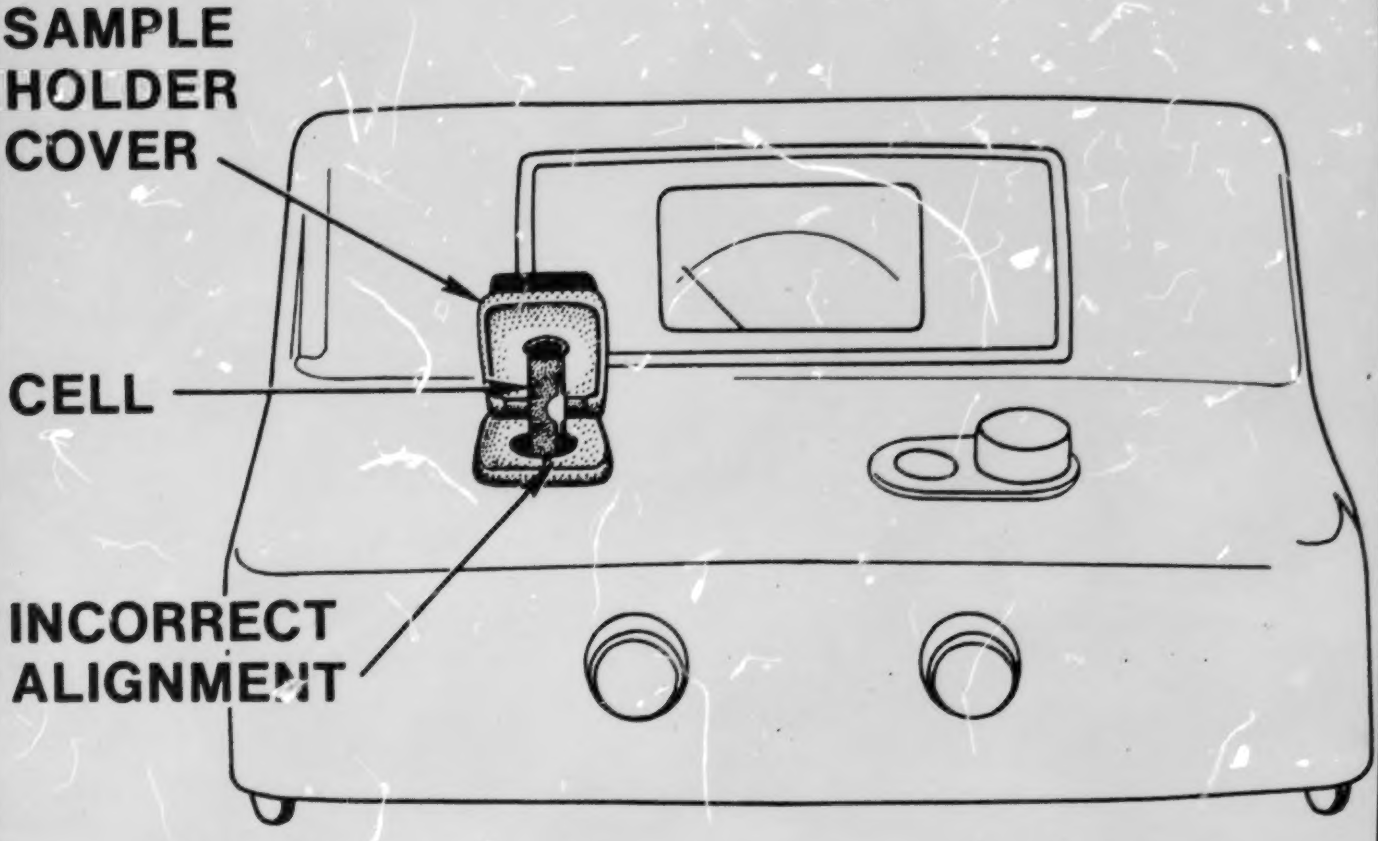


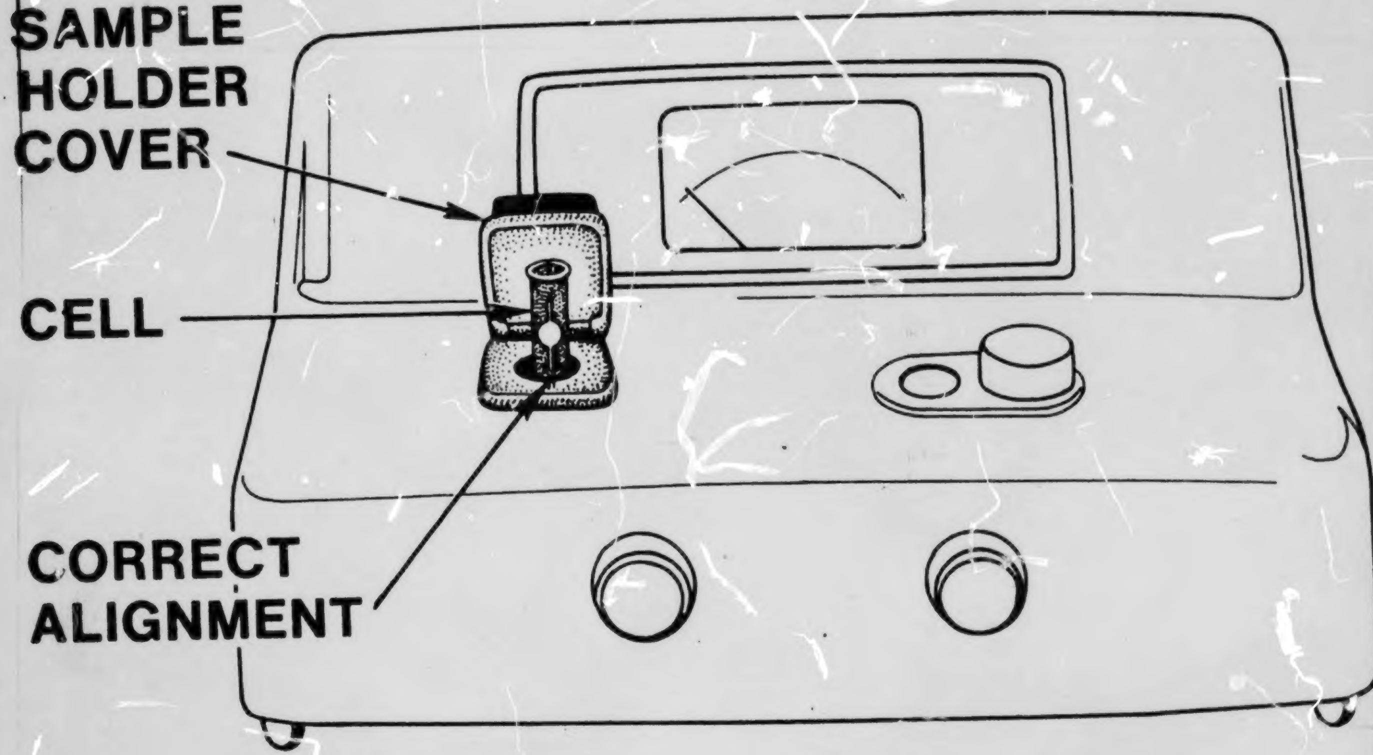
FILTER
FILTER HOLDER

BOTTOM OF SPECTRONIC 20


THUMBSCREW

BOTTOM OF SPECTRONIC 20


SAMPLE
HOLDER
COVER
CELL
INCORRECT
ALIGNMENT


SAMPLE HOLDER COVER
CELL
CORRECT ALIGNMENT

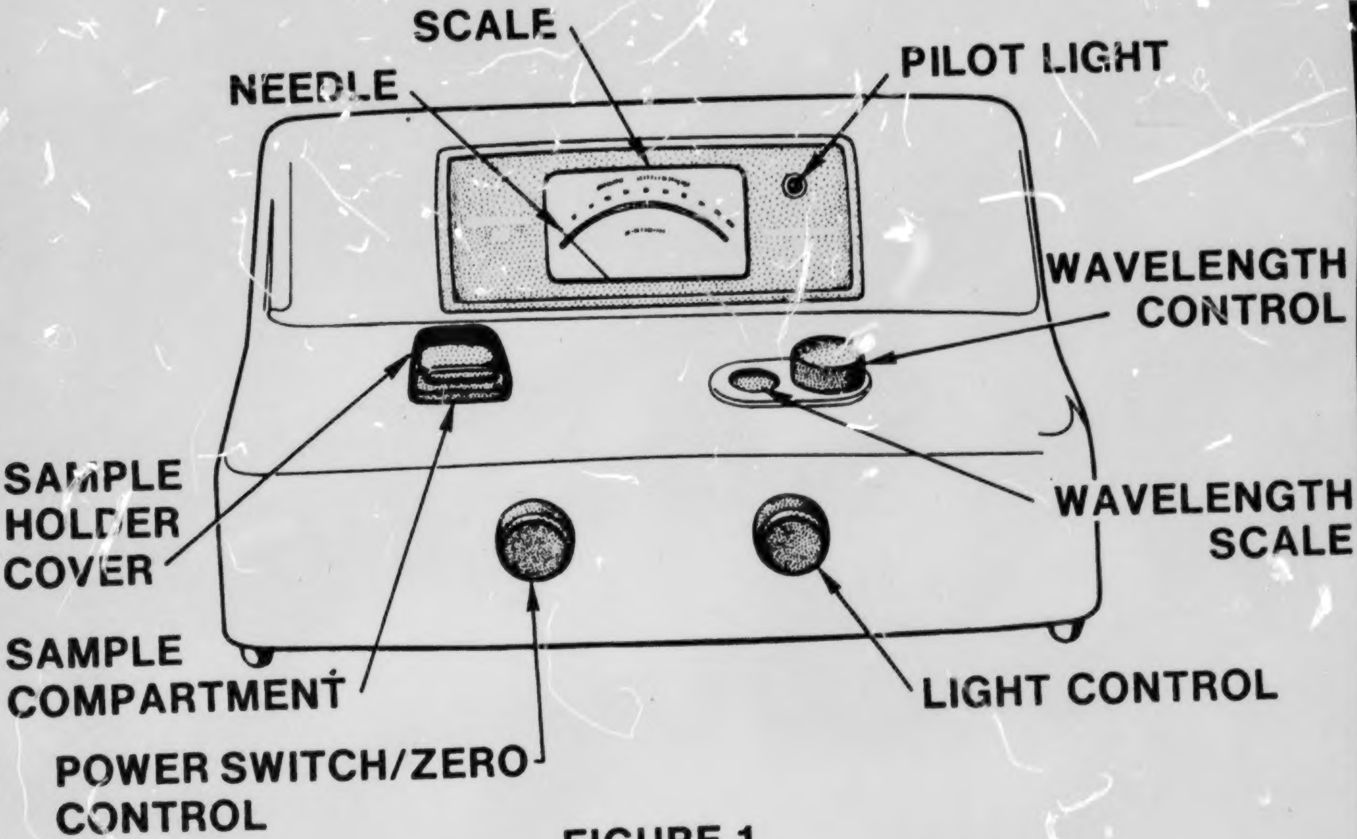

SCALE
PILOT LIGHT
NEEDLE
WAVELENGTH CONTROL
SAMPLE HOLDER COVER
WAVELENGTH SCALE
SAMPLE COMPARTMENT
LIGHT CONTROL
POWER SWITCH/ZERO CONTROL

FIGURE 1

Module No:	**Module Title:** Basic Laboratory Skills
	Submodule Title: Chemistry Skills
Approx. Time: 1 hour	**Topic:** Standard Curves

Objectives:

Upon completion of this module, the participant should be able to:

1. Prepare a standard curve by plotting absorbance vs. concentration of standard solutions and use the standard curve to determine the concentration of an unknown sample given its absorbance.

Instructional Aids:

Handout: "Standard Curves

Instructional Approach:

Lab

References:

Effluent Monitoring Procedures, Nutrients

Class Assignments:

Read handout

Module No:	Topic: Standard Curves

Instructor Notes:	Instructor Outline:
	1. a. Discuss the use of standard curves.
Handout Standard Curves	b. Demonstrate the make up of the curve and use of it to determine the concentratio of a solution.
	c. Work handout
	d. Have participants make up a series of known standards and determine the concentration of an unknown using techniques from the topics Colorimetric Analysis and Standard Curves.

Preparation of Calibration Graphs

1. Analysis Objectives:

   The learner will prepare a calibration graph and will use it to determine the concentration of a chemical constituent in a sample of sewage effluent. The word concentration means how much of the chemical constituent is present in a certain amount of sample; 1.0 milligram/liter is an example value of concentration.

2. Brief Description of Analysis:

   In the field of water pollution analysis, calibration graphs are commonly used in two areas: Absorbance and transmittance measurements. In the first case, energy is absorbed by some chemical constituent in a solution. The amount of energy absorbed or transmitted can be related to the quantity of chemical constituent in a water sample by means of a calibration graph. Examples of absorbance measurements are colorimetric determinations, such as nitrate or phosphate using a spectrophotometer, and the determination of mercury or iron using atomic absorption. Examples of transmittance measurements are the determinations of sodium or potassium using flame photometry.

   Two things must be done in order to prepare a calibration graph. A series of standards must be prepared. A standard is a solution which contains a known amount of the same chemical constituent which is being determined in the sample. Secondly, the absorbance or transmittance of these standards must be measured.

   In order to actually determine how much of the chemical constituent is in the sample, the absorbance or transmittance of the sample must first be determined. The amount of chemical constituent is then read from the calibration graph.

For the sake of simplifying the instruction, absorbance values only will be used in the following procedure.

A. Graph Paper

1. General Comments
    - a. Remove the page containing figure 1.
    - b. Lay it on a desk or any other place where it will be convenient for you to write on it.
        1. For the remainder of this procedure, you will actually use figure 1 and some example absorbance and concentration values to prepare a calibration graph. Additional figures are also included to demonstrate the instructions.
        2. You will have to furnish your own piece of graph paper when you want to prepare other calibration graphs.
2. Labeling the graph paper
    - a. Draw two lines on figure 1 so that it looks like figure 2.
        1. Use a pencil, since you may have to do some erasing during the preparation of the calibration graph.
    - b. Label figure 1 so that it looks like figure 3.
        1. mg/l stands for milligrams per liter. It is an expression of concentration. If the amount of chemical constituent present in the sample is extremely small, the label mg/l

(micrograms per liter) might be used. A stands for absorbance.

2. The mg/l line is a horizontal line. It is called the X axis, or abscissa. The A line is called the Y axis, or ordinate.

c. Examine the example absorbance and concentration values in the column below.

1.

mg/l	A
0.0	0.000
5.0	0.060
10.0	0.120
20.0	0.250
30.0	0.340
40.0	0.470
50.0	0.590

A of sample = 0.180

2. It is data for a series of standards.

3. Each pair of values (e.g. 5.0 and 0.060) represents a point on the graph.

4. Later, you will complete the calibration graph by drawing a straight line through the seven points.

d. Note that the mg/l value is 0.0 and the highest is 50.0.

e. Mark the mg/l axis on figure 1 so that it looks like figure 4.

1. Note that the entire length of the mg/l axis was used. Always use as much of this line as is convenient. Do not, for example, use only one-half of the mg/l axis to mark off the values.

2. Also note that each of the large squares is marked as a whole number of mg/l.

3. Two of the smaller squares equal 1 mg/l.

f. Note that the lowest A value is 0.000 and the highest is 0.590.

1. It is generally not considered good practice to have A values greater than 0.6 or 0.7.

g. Mark the A axis on figure 1 so that it looks like figure 5.

1. Note that the entire length of the A axis was used. Always use as much of this line as convenient. Do not, for example use only one-half of the A axis to mark off the values.

2. Also note that each of the large squares is marked as a whole number of A units.

3. One of the smaller squares equals 0.01 A units.

4. If transmittance measurements were being made, the Y axis or ordinate, would be marked T. T axes are always marked from 0 bottom of axis) to 100 (top of axis).

3. Drawing the calibration graph

a. On figure 1 draw a vertical line from the 50.0 mg/l point of the mg/l axis to the top of the graph.

1. Figure 1 should now look like figure 6.

b. On figure 1 draw a horizontal line from the 0.590 point of the A axis to the right side of the graph.

1. Figure 1 should now look like figure 7.

2. The intersection of these two lines is the point represented by a concentration of 50.0 mg/l and an absorbance of 0.590.

c. Using the same technique as in 1 and 2 above, locate the next five points on figure 1.
   1. The point located at 0.0 and 0.000 is at the intersection of the mg/l and A axes.
   2. Your graph should now look like figure 8. Some analyses may require more than five points.

d. Lay your ruler on figure 1.
   1. So one end of it lies at the 0.0 - 0.000 point, and at the 50.0 - 0.590 point.

e. Look along the edge of the ruler.
   1. The other five points (represented by the intersections of the horizontal and vertical lines do not all lie along the edge of the ruler.

f. Draw a line between the 0.0 - 0.000 and the 50.0 - 0.590 points.
   1. Note that some of the points lie slightly above the line, some lie slightly below the line, and some lie on the line. If one point is considerably off the line, some error in preparing the particular standard was probably made.
   2. This is the line of best fit for the seven points. Always draw the line of best fit when preparing calibration graphs.
   3. The calibration graph is now complete.
   4. Figure 1 should now look like figure 9.
   5. After you have prepared a few calibration graphs, you will find that you won't have to draw the horizontal and vertical lines to locate the points. You'll be able to move your pencil

along the graph paper and put dots at the appropriate points. You'll then draw the line of best fit through them to the 0.0 - 0.000 point.

4. Determining the concentration of the chemical constituent in the sample.
   a. Locate 0.180 on the A axis.
      1. This was the absorbance of the sample
   b. Draw a horizontal line to the right side of the paper.
      1. It should now look like figure 10.
   c. Locate the intersection of this horizontal line and the sloping calibration graph.
   d. From this intersection, draw a vertical line down to the bottom of the paper.
      1. It should now look like figure 11.
   e. Note that the vertical line crosses the mg/l axis at 15.3
      1. Recall that on the mg/l axis, 2 of the small squares equal 1 mg/l.
      2. 15.3 mg/l is therefore the concentration of the chemical constituent being measured in the sample.
5. Sample dilution
   a. If it was necessary to dilute the sample, the value read from the mg/l axis must be multiplied by a dilution factor.
      1. The dilution may have been necessary so that the A value for the sample would not be greater than the A value obtained for the highest concentration standard; 0.590 in this set of example data.

2. The dilution factor is the ml. of sample taken for dilution, divided into the ml. to which it was diluted; e.g., if 10.0 ml. of the original sample were diluted to 1000 ml. (as in a volumetric flask) the dilution factor would be 1000/10, or 100/
3. In some determinations, you may prepare more than one dilution of the sample. Look at the mg/l axis of figure 1 and assume that three dilutions of the sample gave values of 2.2, 24.0, and 48.0 mg/l, before correcting for the dilution factor. It is common practice to use the 24.0 value, since it lies nearest the middle of the calibration graph.

FIGURE 1

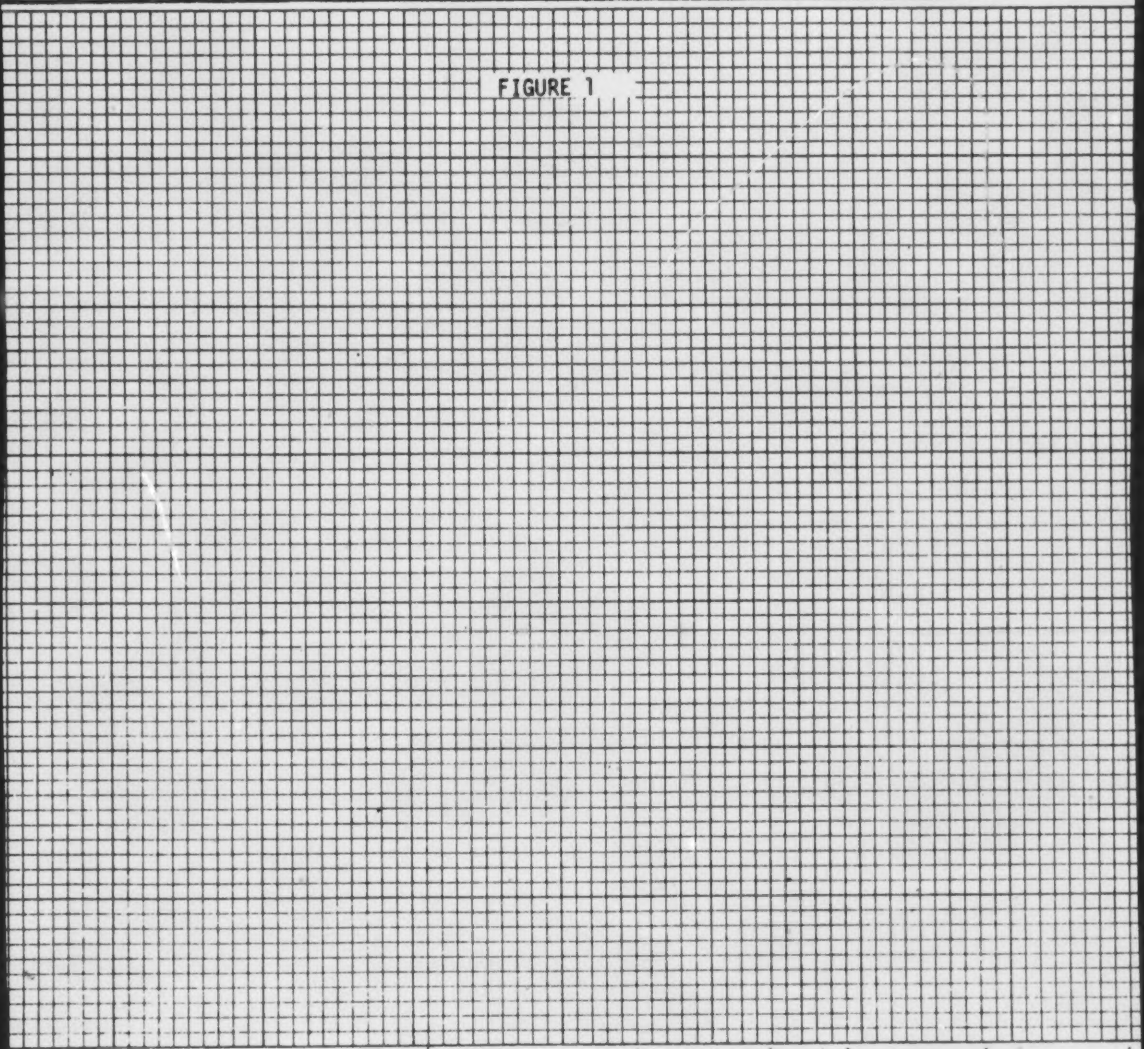


FIGURE 2

FIGURE 3

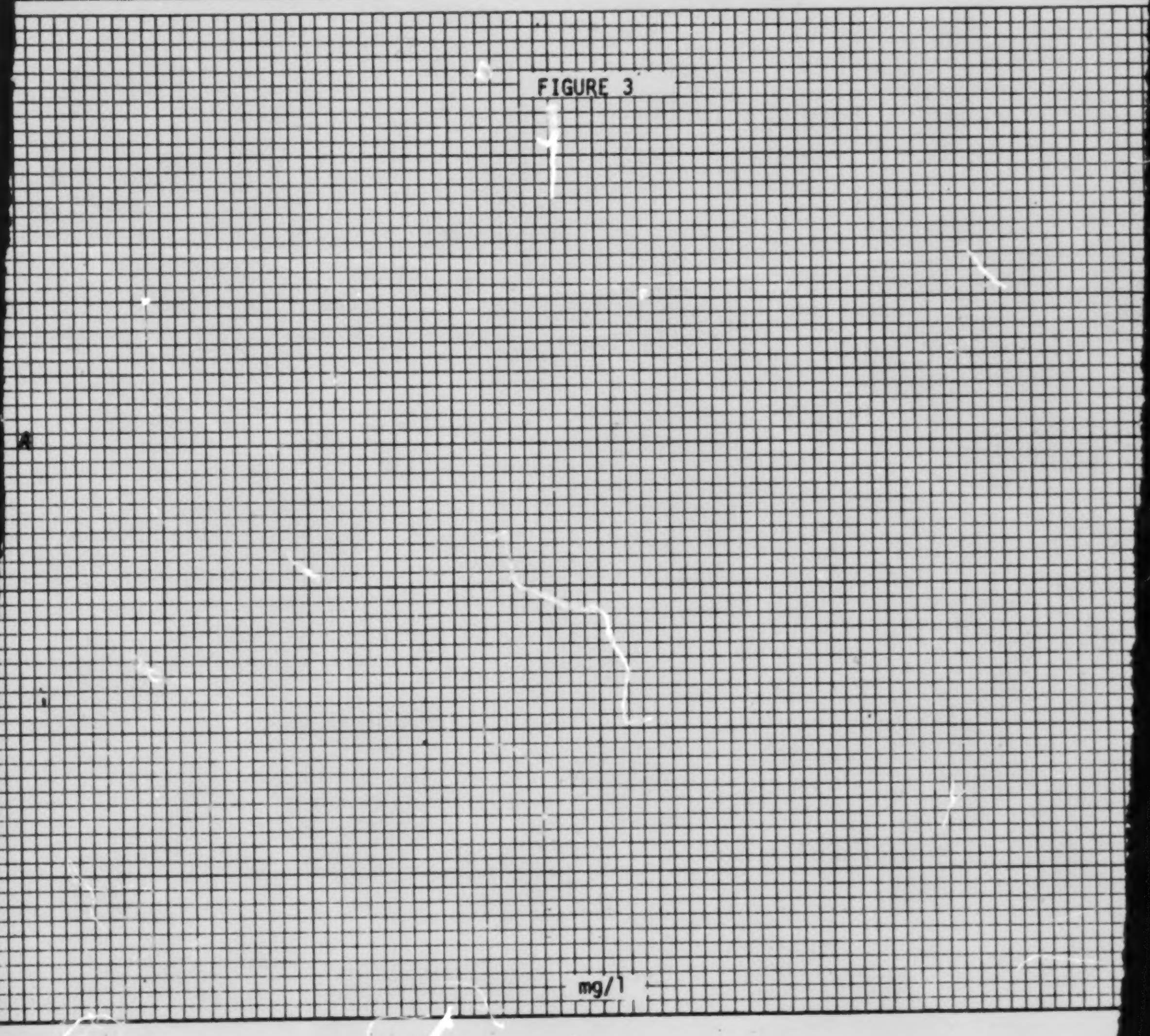


FIGURE 4

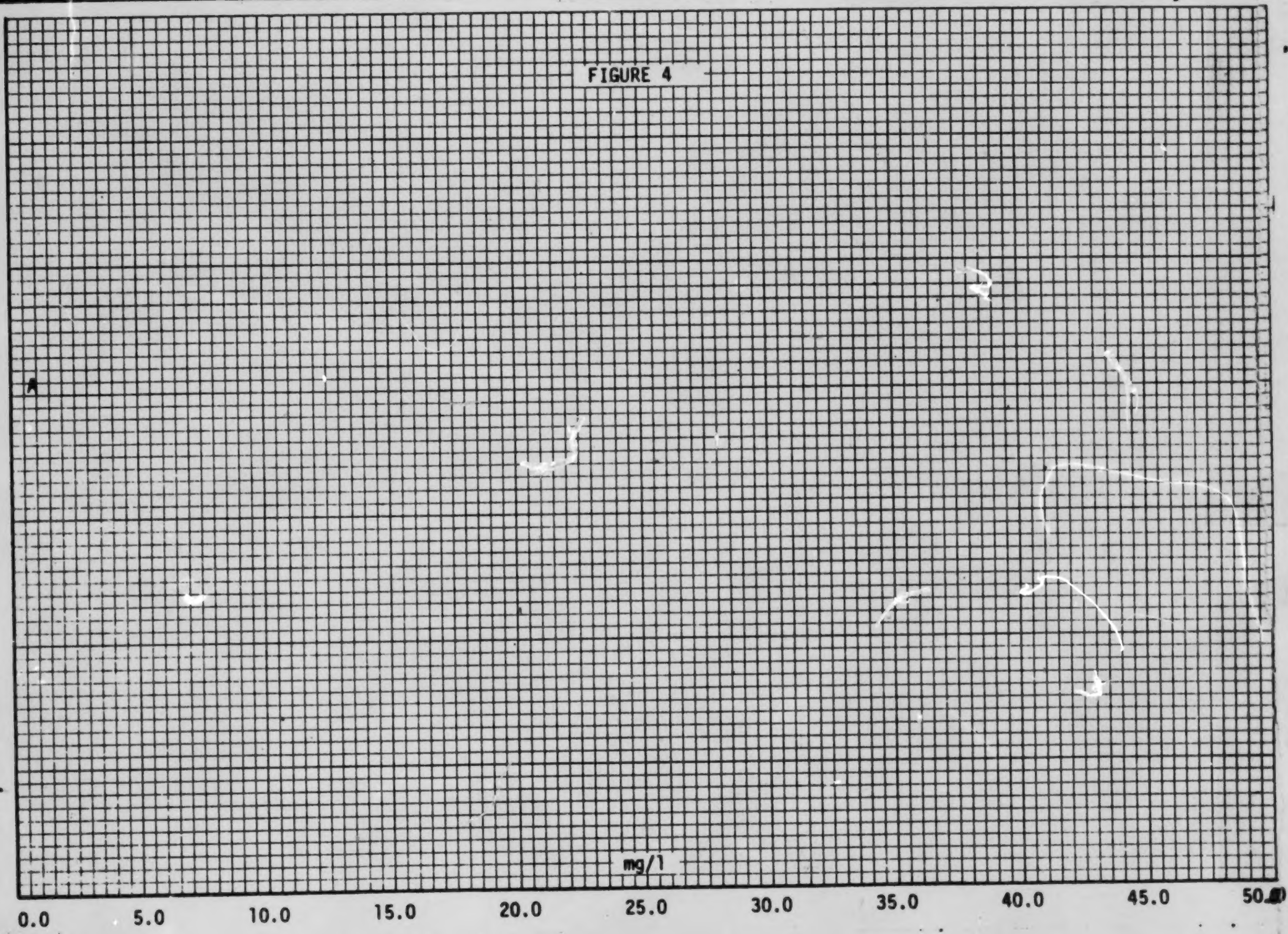


FIGURE 5

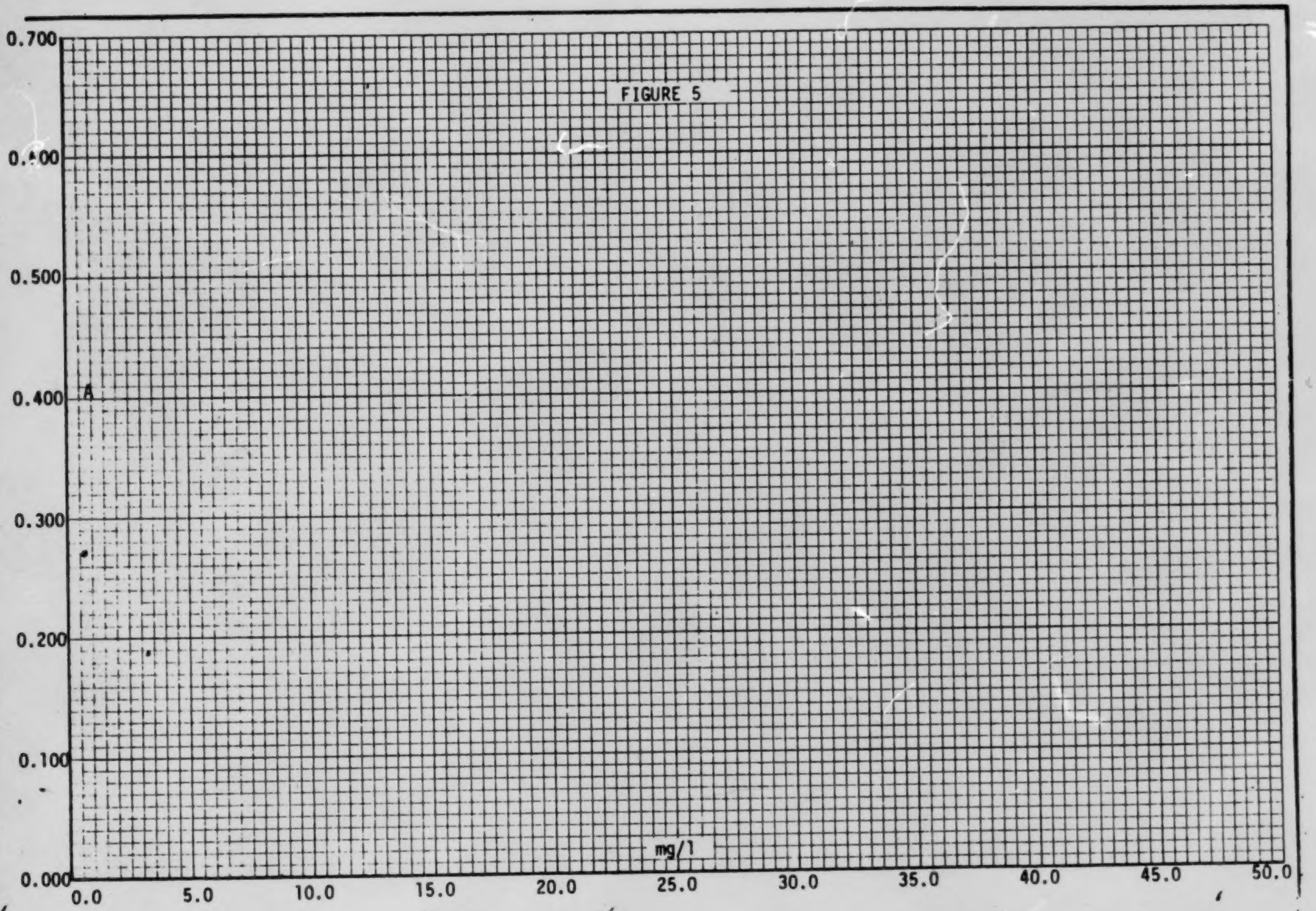

0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
A
mg/l
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
45.0
50.0

FIGURE 6

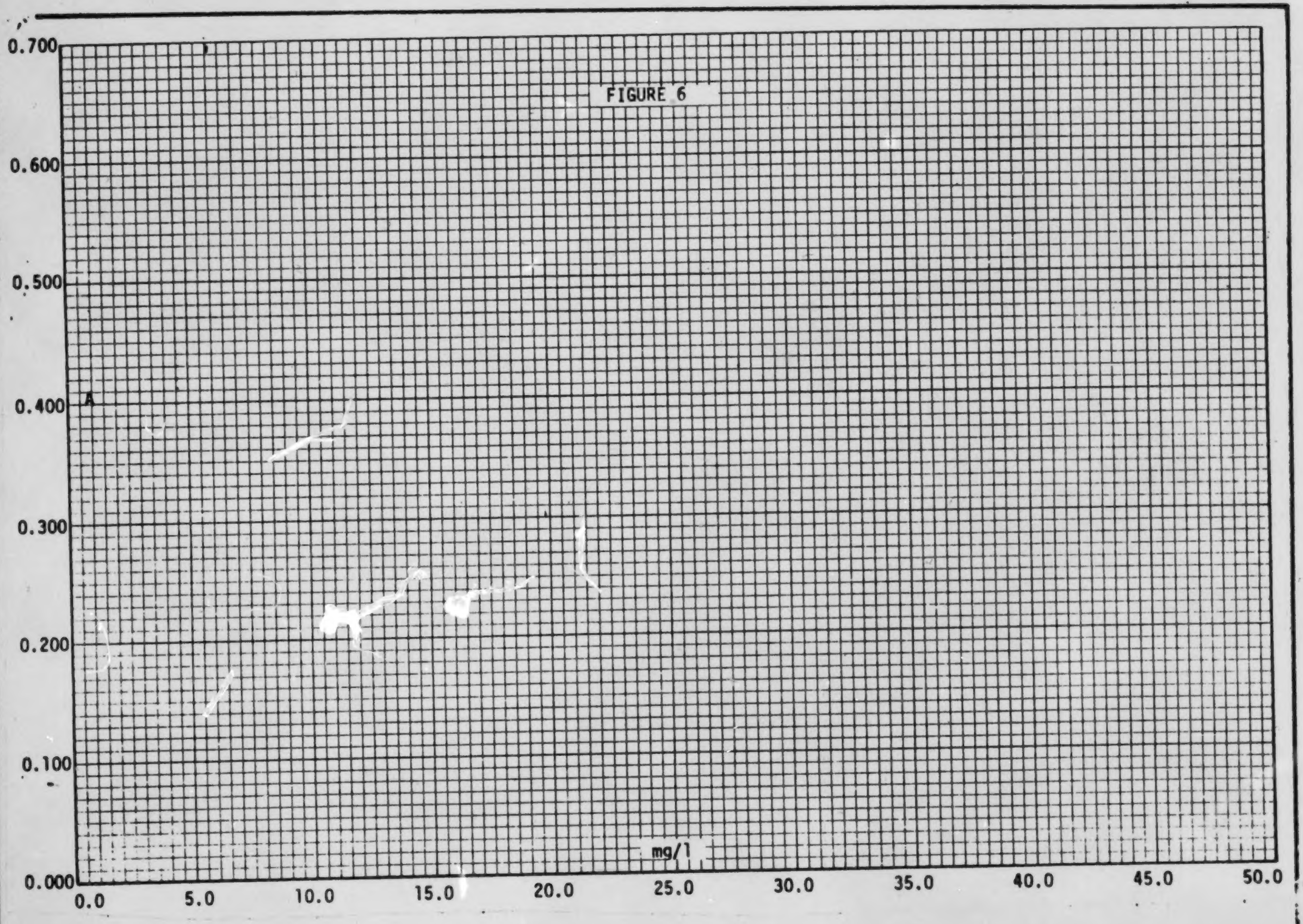

0.700
0.600
0.500
0.400
A
0.300
0.200
0.100
0.000
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
45.0
50.0
mg/l

FIGURE 7

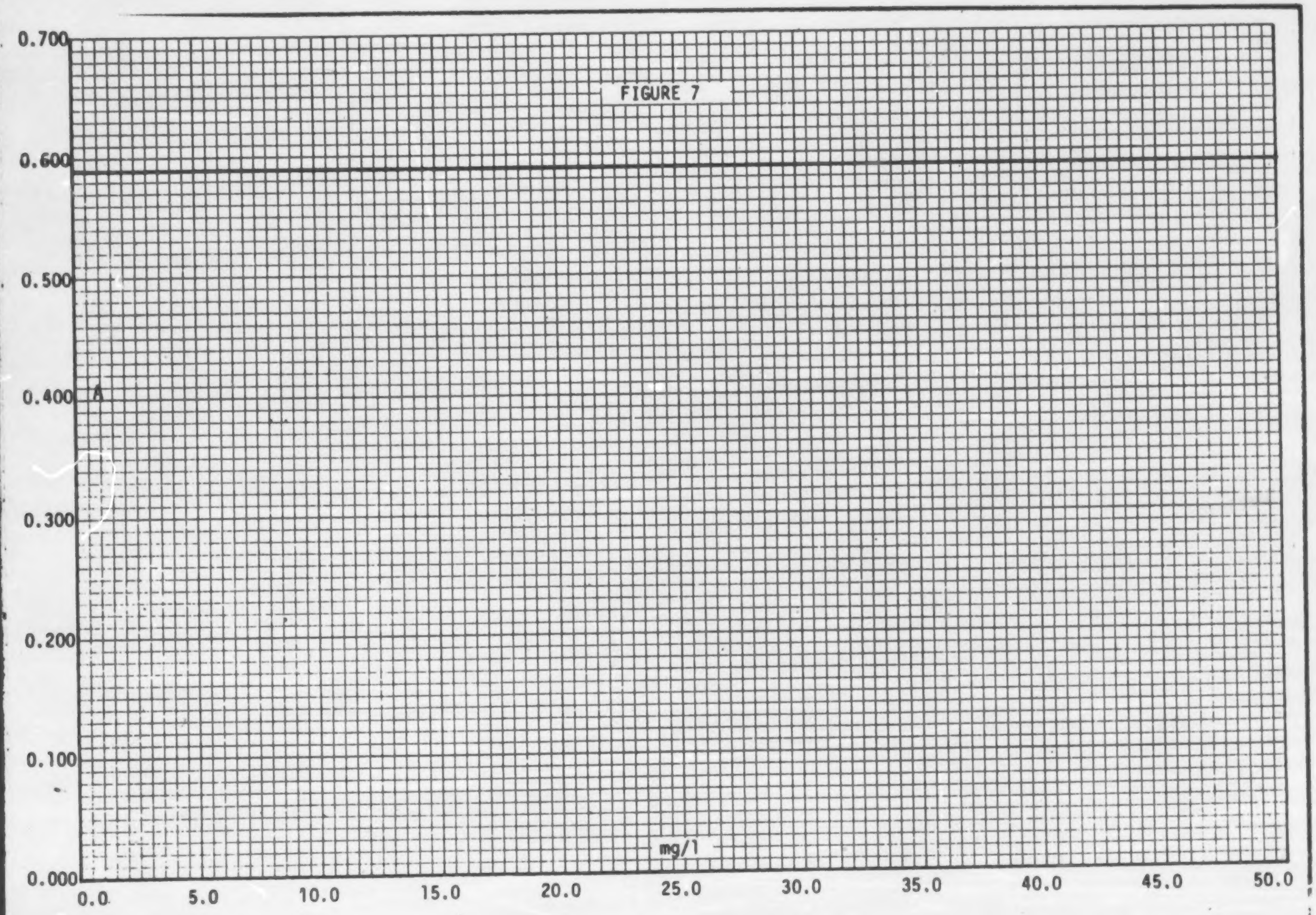
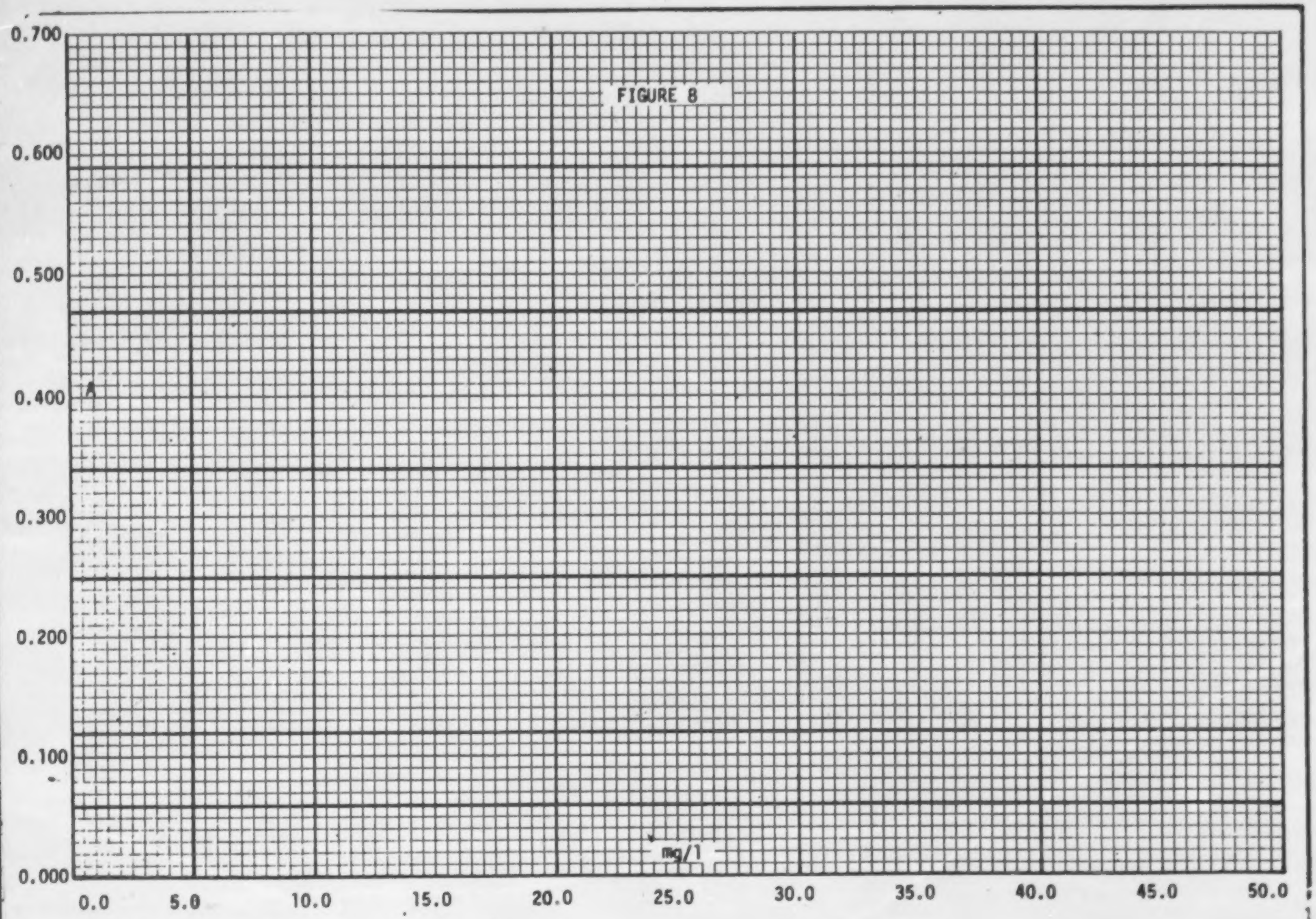

0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
A
mg/l
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
45.0
50.0

FIGURE 8


0.700
0.600
0.500
0.400
A
0.300
0.200
0.100
0.000
mg/l
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
45.0
50.0

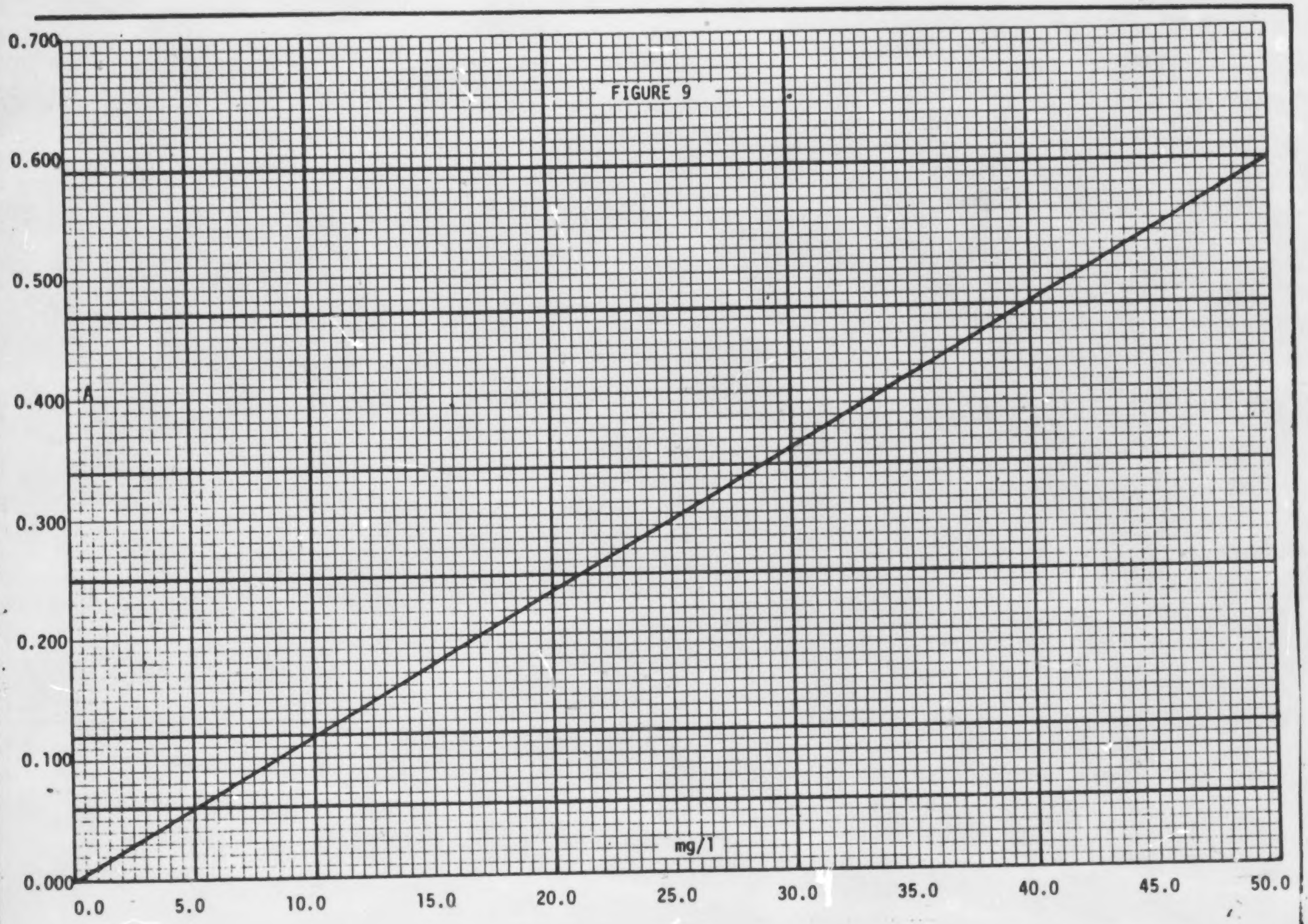
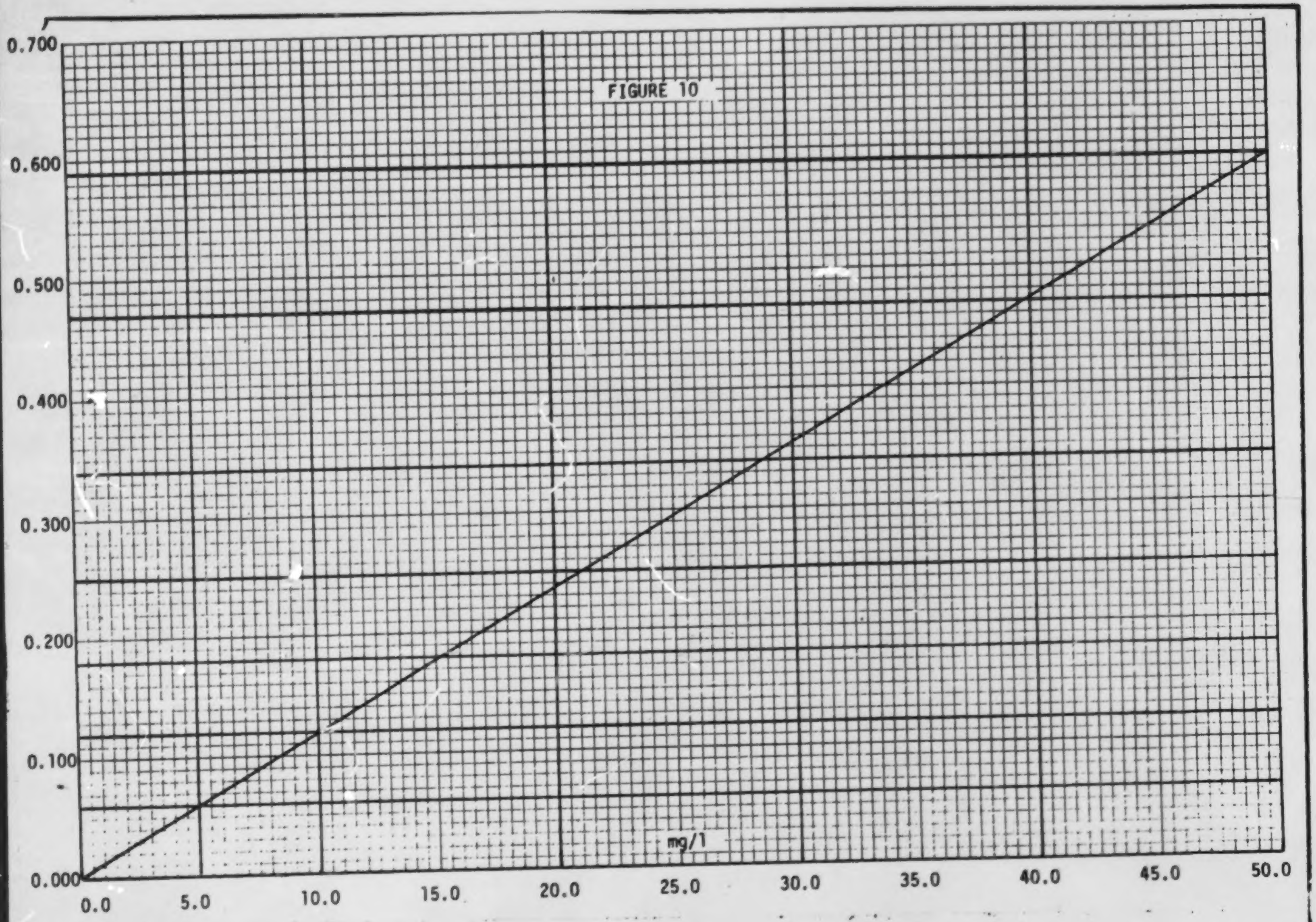


FIGURE 9

0.700
0.600
0.500
0.400
A
0.300
0.200
0.100
0.000
mg/l
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
45.0
50.0

FIGURE 10


0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
45.0
50.0
mg/l

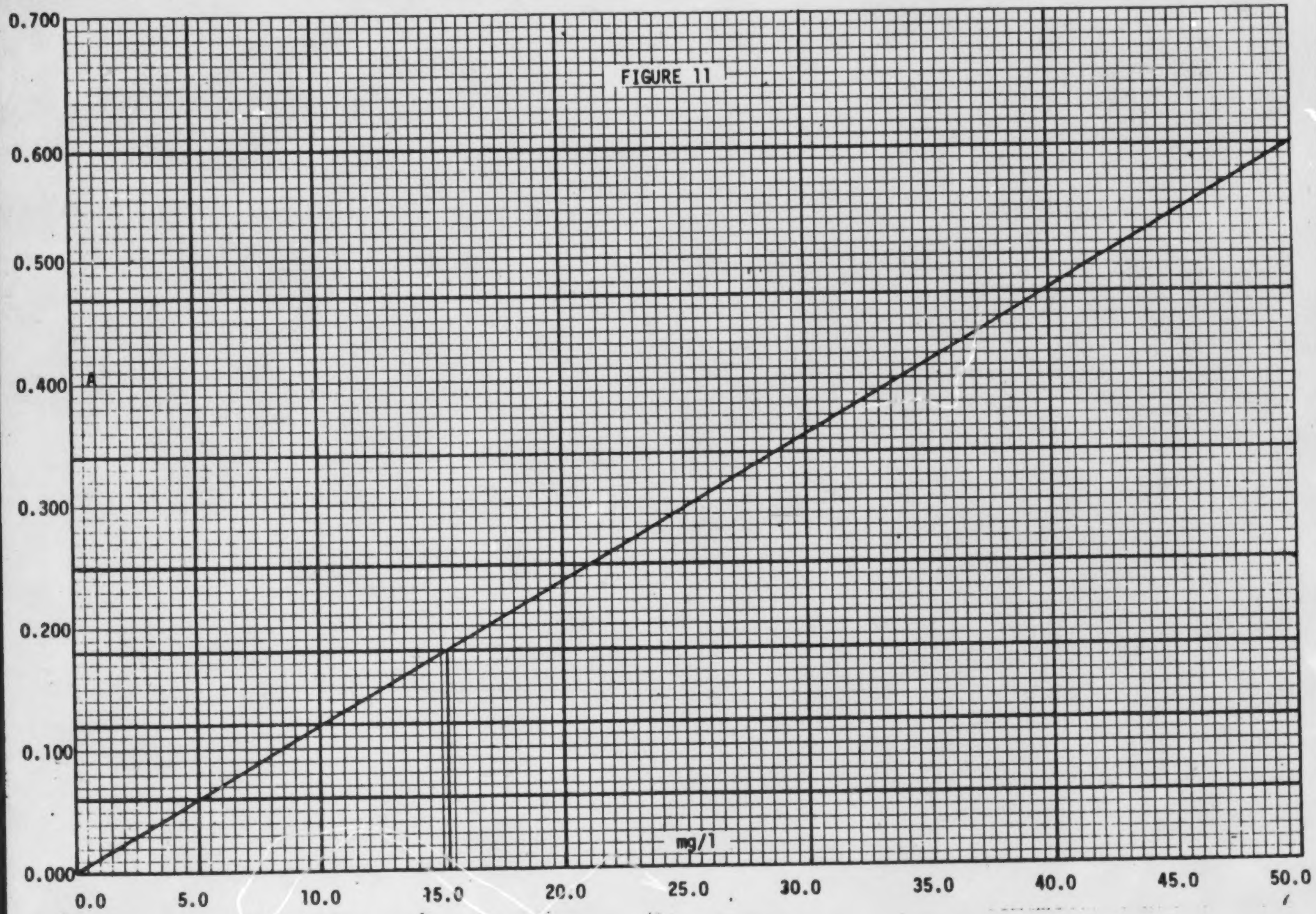


FIGURE 11

A
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
mg/l
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
45.0
50.0

Module No:

Module Title:
Basic Laboratory Skills

Submodule Title:
Chemistry Skills

Approx. Time:
1 hour

Topic:
Lab Supplies and Chemicals

Objectives:

Upon completion of this module, the participant should be able to:

1. Demonstrate the use of lab supply and chemical catalog in procuring lab supplies and chemicals.

Instructional Aids:

Catalogs Chemical and Supply

Instructional Approach:

Lab
Lecture

References:

None

Class Assignments:

Participate in laboratory practice session

Module No:_	Topic: Lab Supplies and Chemicals

Instructor Notes:	Instructor Outline:
	1. a. Discuss lab and chemical supply catalogs. Grade of glassware, chemicals and equipment. Discuss shelf life and quantities to be ordered. b. Given a list of chemicals and apparatus have participant make up an order.

Module No:

Module Title:
Basic Laboratory Skills

Submodule Title:
Chemistry Skills

Approx. Time:
30 Min.

Topic:
Standard References

Objectives:

Upon completion of this module, the participant should be able to:

1. List the standard references approved by the Environmental Protection Agency.

Instructional Aids:

All Standard References

Instructional Approach:

Lecture

References:

Federal Regulations, Vol. 28, No. 199, pt2, Oct. 16, 1973

Class Assignments:

None

Module No:	Topic: Standard References

Instructor Notes:	Instructor Outline:
	List the standard references used in water and wastewater labs. Compare the formats of the references. Discuss the use of non-standard methods along with the value of standard methods.

Module No:

Module Title:
Basic Laboratory Skills

Submodule Title:
Microbiology Skills

Approx. Time:
1/3 hour

Topic:
Laboratory Cleanliness

Objectives:

Upon completion of this module, the participant should be able to:

1. State the proper method of cleaning a laboratory.
2. Identify the proper schedule and reason for laboratory cleaning.

Instructional Aids:

Handout: Laboratory Cleanliness

Instructional Approach:

Lecture
Discussion

References:

1. Standard Methods for the Examination of Water and Wastewater, 14th Edition.

Class Assignments:

Read handout

Module No:

Topic: Laboratory Cleanliness

Instructor Notes:	Instructor Outline:
Handout: Laboratory Cleanliness	
1. Include discussion of: a. Disinfectants b. Use of vacuum cleaners c. Cleaning tools 1. Sponges 2. Towels 3. Scrubbers d. Cleaning and preserving stainless steel with mineral oil	1. Discuss methods of cleaning a laboratory used for microbiological analysis.
2. Include discussion of: a. Daily wipedowns b. Weekly wipedowns c. Major cleaning days d. Garbage cleanup	2. Discuss cleaning schedules and rational behind the schedules.

LABORATORY CLEANLINESS

A. Types of disinfectants

  1. 70% Ethanol
  2. Phenols i.e. O-Syl
  3. Quaterniary ammonium compounds
  4. Halogen compounds
  5. Activated sialdehyde i.e. cidex

B. Use of disinfectants

  1. Weekly
    a. Wipe down all shelves removing all glassware and books
    b. Wipe down all incubators, inside and outside
    c. Wipe out inside of autoclave.
  2. Daily
    a. Wipe down tops of all counters, large pieces of equipment
  3. Immediately before testing disinfect work area
  4. Immediately disinfect spills

C. Sources of Contamination

  1. Dirt around lab
  2. Spilled samples or cultures
  3. Un-autoclaved bacterial garbage
  4. Chemical contamination from use of glassware for both Chemistry testing and Bacterial testing.

Module No:

Module Title:

Basic Laboratory Skills

Submodule Title:

Microbiology Skills

Approx. Time:

2/3 hour

Topic:

Equipment Packaging

Objectives:

Upon completion of this module, the participant should be able to:

1. Demonstrate the ability to determine how a piece of equipment must be packaged and labeled for sterilization.
2. Identify reason for packaging equipment.

Instructional Aids:

Handout: Equipment Packaging
Laboratory practice

Instructional Approach:

Lecture
Discussion
Demonstration and supervised laboratory practice

References:

1. Standard Methods for the Examination of Water and Wastewater, 14th Edition.

Class Assignments:

Read handout
Complete laboratory assignment

Module No:	Topic: Equipment Packaging

Instructor Notes:	Instructor Outline:
Handout: Equipment Packaging 1. Include explanation of: a. Why brown (non-bleeding) Kraft paper is used. b. When aluminum foil is used. c. What a bacterial barrier represents. 1. In liquid 2. In air	1. Discuss and demonstrate the choice and method of equipment packaging including packaging for steam and hot air sterilization.
	2. Discuss the purpose of the packaging.
	3. Have participant practice by packaging an article for sterilization.

EQUIPMENT PACKAGING

I. Preparation
    A. All glassware and filter funnels must be thoroughly washed in non-toxic detergent
        1. i.e. Alconox
        2. Removes bacterial scum from glassware
    B. Rinse 6 - 12 times in hot tap water
        1. Removes detergent residue
        2. Residue is harmful to bacteria
    C. Final rinse 1 - 3 times in distilled water
        1. Removes mineral residue from tap water
        2. Prevents water spotting
    D. Air Dry
        1. Any spot indicates dirt
        2. Rewash before using
II. Packaging
    A. Reasons for packaging
        1. Creates a bacteria barrier
        2. Allows for storage of sterile equipment
    B. Proper labeling
        1. Define contents
        2. Date to aid in equipment rotation

C. Proper package
    1. Brown Kraft paper
    2. Aluminum foil
    3. Glycine bags
    4. Misc. containers appropriate to sterilization method

III. Sterilization of equipment - 2 Acceptable Methods

A. Autoclave
    1. All rubber, metal and glassware and some plastics
    2. Normal cycle 15 min. 15 121° C.
    3. Exhaust rapidly

B. Hot air sterilizing oven
    1. Dry glassware and metal objects only
    2. Normal cycle 1 hr. at 170° C.
    3. Allow to cool before use
    4. Package pipets in metal containers
    5. Package other equipment with aluminum foil

Module No:

Module Title:
Basic Laboratory Skills

Submodule Title:
Microbiology Skills

Approx. Time:
2/3 hour

Topic:
Media and Reagent Preparation

Objectives:

Upon completion of this module, the participant should be able to:

1. Demonstrate the ability to prepare and dispense microbiologicals.
2. State precautions which must be taken to insure accuracy.

Instructional Aids:

Laboratory Practice

Instructional Approach:

Lecture
Discussion
Demonstration and laboratory practice

References:

1. Standard Methods for the Examination of Water and Wastewater, 14th Edition.

Class Assignments:

Complete laboratory assignment

Module No:

Topic:
Media and Reagent Preparation

Instructor Notes:	Instructor Outline:
1. Emphasize: a. Complete dissolution b. Proper heating c. Accurate dispensing d. Careful sterilization	1. Discuss and demonstrate the proper procedure for preparation and dispensing microbiologicals.
2. Include: a. Measurement b. Overheating c. Under heating d. Sterilization	2. Describe areas of common error and discuss precautionary measures.

Module No:

Module Title:

Basic Laboratory Skills

Submodule Title:

Microbiology Skills

Approx. Time:

1/2 hour

Topic:

Autoclaves & Steilizing Ovens

Objectives:

Upon completion of this module, the participant should be able to:

1. State precautions applicable to the use and care of all autoclaves and sterilizing ovens.
2. Demonstrate the proper loading, cycling, and removal of sterile equipment from an autoclave and sterilizing oven.
3. Differentiate between items sterilized in an autoclave and those sterilized in a sterilizing oven.

Instructional Aids:

Handout
Laboratory Practice

Instructional Approach:

Lecture
Discussion

References:

1. Standard Methods for the Examination of Water and Wastewater, 14th Edition.

Class Assignments:

Read handout
Complete laboratory assignment

Module No:	Topic: Autoclaves and Sterilizing Ovens

Instructor Notes:	Instructor Outline:
Handout: Autoclaves and Sterilizing Ovens	
1. Emphasize safety	1. Discuss the precautions which must be taken when operating: a. An autoclave b. Sterilizing oven
	2. Describe care and cleaning procedures for autoclaves and sterilizing ovens.
	3. Describe and demonstrate the proper loading and use of autoclaves and sterilizing ovens.
	4. Describe the type of equipment which is sterilized by each of the methods discussed.

AUTOCLAVES AND STERILIZING OVENS

A. Autoclave

1. Before using read and follow manufacturers installation use and maintenance instructions and safety precautions.

2. Normal sterilization = 15 psi yielding 121° C. for 15 min.

3. Use to sterilize liquids and non-heat sensitive equipment

    a. Most plastics are not autoclavable and sterilized by manufacturer.

    b. Sterilized media and reagents must be removed from autoclave as soon as possible after autoclave is opened.

    c. Glassware may be sterilized in autoclave but must be allowed to dry before removing from autoclave.

B. Hot air Sterilizing Oven

1. Before using read and follow manufacturers installation, use, and maintenance instructions and safety precautions.

2. Normal Sterilization = 1 hour at 180° C.

3. Use to sterilize glass and metal only

    a. Rubber and plastics will melt.

    b. Liquids will evaporate and grow media components will be destroyed

Module No:	**Module Title:** Basic Laboratory Skills
	Submodule Title: Microbiology Skills
Approx. Time: 1½ hour	**Topic:** Microscopes

Objectives:

Upon completion of this module, the participant should be able to:

1. State precautions applicable to the care and use of microscopes.
2. Identify and use a microscope to focus a speciman given the microscope, the speciman and appropriate reference materials.

Instructional Aids:

Handout: Microscopes
Transparancy on Microscopes
Laboratory practice

Instructional Approach:

Lecture
Discussion

References:

1. Standard Methods for the Examination of Water and Wastewater, 14th Edition
2. Benson, Harold, Microbiological Applications, Wm. C. Brown Inc., Dubuque, Iowa, 1967.

Class Assignments:

Read handout
Complete laboratory assignment

Module No:	Topic: Microscopes

Instructor Notes:	Instructor Outline:
Handout: Microscopes	
Microscope Transparancy	
1. Include:	1. Discuss and demonstrate proper care of a microscope.
a. Handling	
b. Storage	
c. Cleaning	
	2. Discuss and demonstrate the proper method of focusing and examining a speciman
	a. Using a compound microscope
	b. Using a dissecting microscope
	3. Differentiate between a compound microscope and a dissecting microscope by examining the components of each.

MICROSCOPES

Proper Care

Regardless of whether a microscope is a compound or dissecting microscope, they are essentially similar. All contain a controlled light source and a geared mechanism for adjusting the distance between the object and the lenses. When carrying a microscope, always use both hands. Grap the arm with your right hand and use your left hand to grip the base. Carry the scope directly in front of you. If it is allowed to swing at your side, the microscope can easily be damaged by a collision with a door frame or piece of furniture.

Cleaning the lenses must be done with great care as they can be easily scratched and any such mar on the highly polished surface will impare its efficiency. Dust on the eye pieces or objectives should only be removed with lens tissue, a camel's hair brush. Dust inside the eyepiece can be gently blown out. Use lens cleaner (an oil solvent) sparingly on a lens tissue to remove oil from eyelashes on the eyepieces or immersion oil from the oil immersion lens. Quickly remove any excess lens cleaner with a dry lens tissue.

When cleaning the eyepiece be sure and cover the open end with a tissue to keep out any dust.

After use, care must be taken to (1) remove the speciman from the stage, (2) Remove all oil or other debris from stage and lens, (3) Return lenses to low power position, (4) Secure any electrical cords around scope, (5) Re-center stage (If mechanical), (6) Replace dust cover and store in designated cupboard.

Focusing

In focusing the dissecting microscope, simply place the speciman on the stage and adjust the distance with the focusing knob until the speciman is clearly seen.

Focusing a compound microscope is a bit more difficult since you have a series of objectives to work with. To focus for low power (10 x) examination, (1) Raise the condenser to top position and close down diaphram to lower the light level to best see the speciman, (2) Swing the 16 mm. (10 x) lens into position (3) Lower the lens to just above the speciman (B & L) or to stop position (A.O.) and focus by raising objective with fine adjustment knob.

From the focused low power you can go directly to the high dry lens (43 x) with only minor adjustment using the fine knob to bring the speciman into focus.

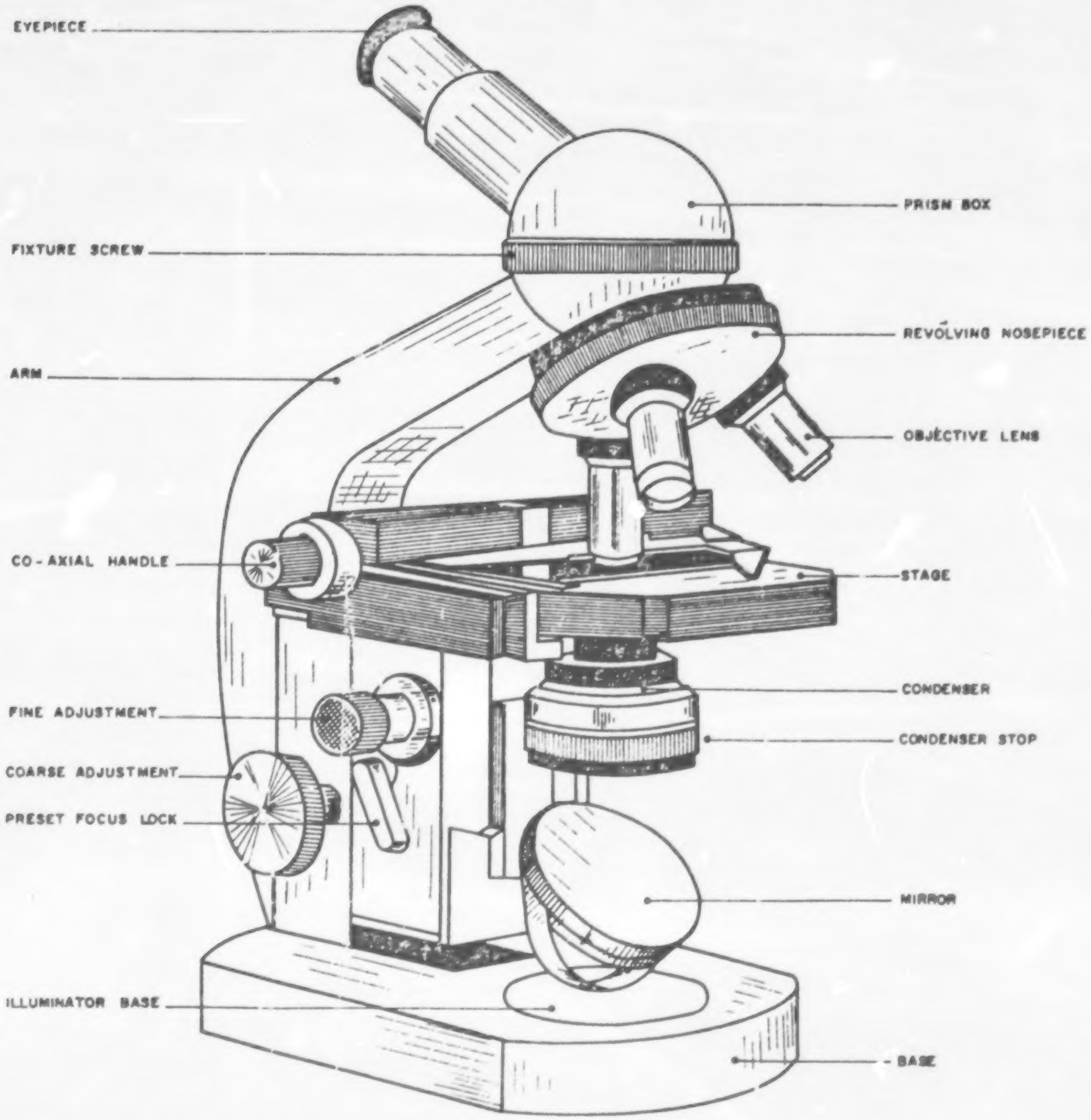
To move, however, to the oil immersion lens (100 x) a drop of immersion oil must be placed between the lens and the speciman. The lens is then lowered to make contact with the oil and then the fine adjustment knob is used to focus the speciman.

The low power lens is primarily used to scan the slide and the high-dry for focusing protozoa, algae and mold. The oil immersion lens is used directly for stained bacteria as the low power and high dry do not magnify sufficiently even for scanning.

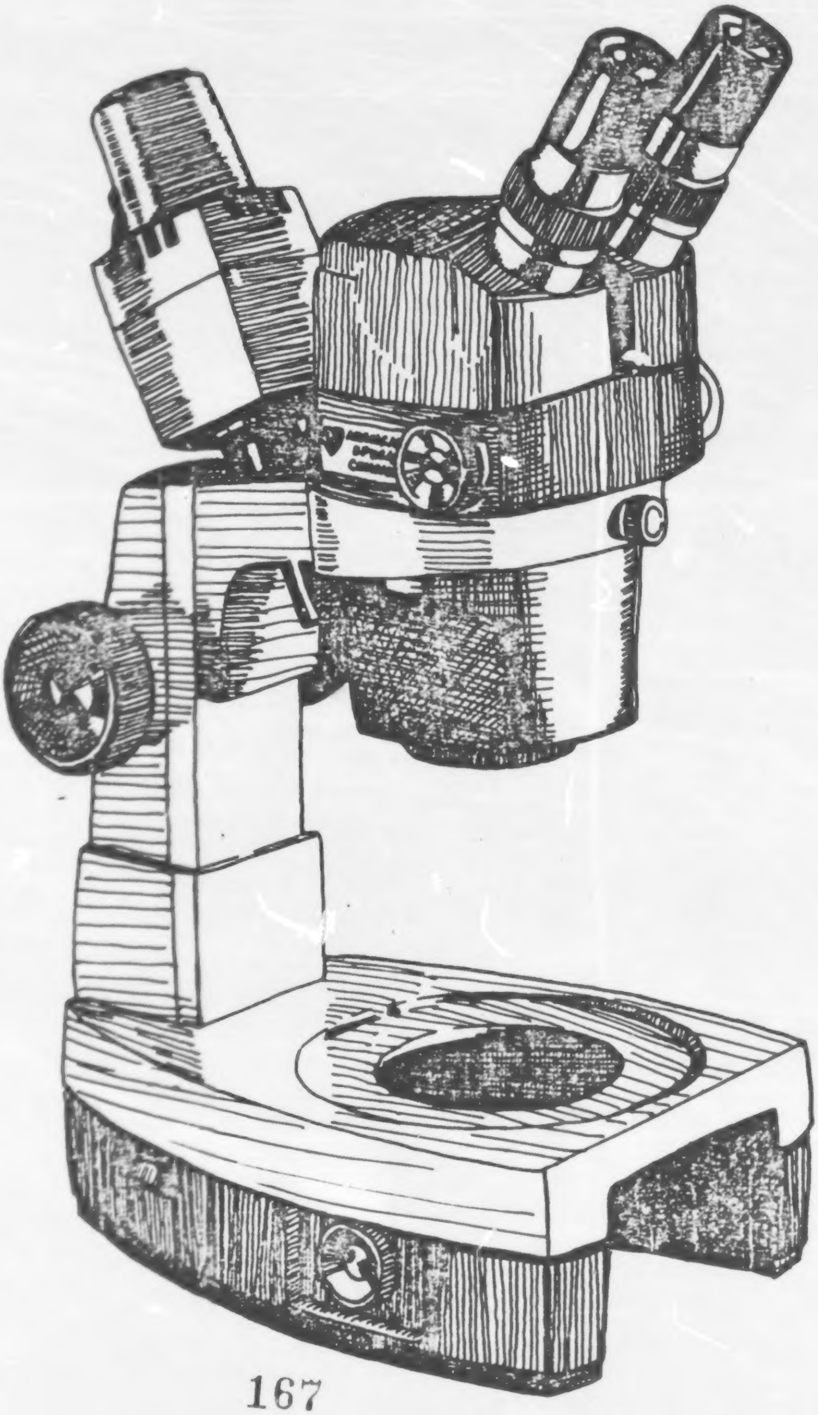
Structure

In order to best compare the differences in structures is to examine the following two diagrams:

PARTS OF THE MICROSCOPE


EYEPIECE
PRISM BOX
FIXTURE SCREW
REVOLVING NOSEPIECE
ARM
OBJECTIVE LENS
CO-AXIAL HANDLE
STAGE
CONDENSER
FINE ADJUSTMENT
CONDENSER STOP
COARSE ADJUSTMENT
PRESET FOCUS LOCK
MIRROR
ILLUMINATOR BASE
BASE

PARTS OF THE DISSECTING SCOPE



Module No:

Module Title:
Basic Laboratory Skills

Submodule Title:
Microbiology Skills

Approx. Time:
1 hour

Topic:
Aseptic Technique

Objectives:

Upon completion of this module, the participant should be able to:

1. Demonstrate aseptic technique in making transfers from bottles and other containers using pipets, loops, and needles and forceps.
2. Identify reasons for aseptic technique in making transfers.

Instructional Aids:

Laboratory Practice

Instructional Approach:

Lecture
Discussion
Demonstration and Supervised Laboratory Practice

References:

Standard Methods for the Examination of Water and Wastewater

Class Assignments:

Complete laboratory assignment

Module No:	Topic: Aseptic Technique

Instructor Notes:	Instructor Outline:
1. Using: a. Pipets b. Loops c. Needles d. Forceps	1. Discuss and demonstrate the proper procedures for making aseptic transfers from: a. Dilution blanks to tubes b. Dilution blanks to filtering funnels c. Tubes to tubes d. Other containers using forceps
	2. Discuss necessity for aseptic technique.
	3. Describe problems which arise when aseptic technique to actual laboratory procedures.
4. Include: a. Sample collection b. Sample dilution c. Media transfers d. Culture transfers	4. Relate aseptic technique to actual laboratory procedures.
5. Include: a. Weighing dry chemicals and biologicals b. Use of dirty glassware c. Storing and measuring solutes (i.e. distilled water)	5. Explain how routine practice of aseptic technique where applicable leads to more awareness of contamination in other areas.

Module No:	**Module Title:** Basic Laboratory Skills
	Submodule Title: Microbiology Skills
Approx. Time: 1/2 hour	**Topic:** Microbiological Sample Collection

Objectives:

Upon completion of this module, the participant should be able to:

1. Properly prepare sample bottle and take a grab sample from:
    a. A spigot or tap
    b. An open body of water
2. Identify precautions which must be taken before, during and after sampling to protect sample and reasons for these precautions.

Instructional Aids:

Handout: Microbiological Sample Collection

Instructional Approach:

Lecture
Discussion

References:

Standard Methods for the Examination of Water and Wastewater

Class Assignments:

Read handout

Module No:	Topic: Microbiological Sample Collection

Instructor Notes:	Instructor Outline:
Handout: Microbiological Sample Collection	1. Describe the proper method of preparing a sample bottle for the collection of microbiological samples from: a. Chlorinated sources b. Unchlorinated sources 2. Describe areas where error is likely to occur and the effect on the final result. 3. Describe the proper procedure for obtaining a grab sample from a spigot or tap and an open body of water. 4. Discuss sample protection and preservation.

MICROBIOLOGICAL SAMPLE COLLECTION

I. Preparation of Sampling Equipment

A. Sample bottles must be:

1. At least 100 ml capacity with a large neck opening.
2. Thoroughly cleaned with detergent, rinsed 6 times in hot tap water, rinsed finally in distilled deionized water, then air dried.
3. Free from spots, scum, chips, cracks, excessive scratches and other damage on which bacteria may lodge.
4. Closed with preferably an all glass ground cap closure (but screw caps can be used providing liners are free from contamination and provide a non-leaking seal.
5. Sterilized in an autoclave at 121^{0} C. for 15 min. with Kraft paper or tin foil hood covering caps and necks of bottles and slip of paper between bottleneck and glass stopper to prevent glass stopper from sticking.

B. Bottles intended for use in collection of chlorinated samples must have a 10% sodium thiosulfate solution added at the rate of 0.1 ml for each 4 oz. bottle prior to sterilization and sterilized in bottle.

C. Labels must be:

1. Clean and unused
2. Attached to bottle by a means not affected by water (i.e. string or wire.)

D. Label markers must be:

1. Permanent type not affected by water
2. Able to mark on label

E. Sampling devices must be in working condition and properly maintained.

F. Germicide must be available to clean up spills but must not come in contact with sample or any equipment touched by sample.

G. Rubber gloves must fit and not be punctured.

H. Ice chest for transporting sample must be:

1. Sufficient size to accommodate all samples
2. Undamaged with tight cover so cold temperature can be maintained inside.
3. Filled with enough ice to quickly chill sample but little or no free water.

I. Refrigerator must be set at 2 - 10° C. and used if samples are not examined upon immediate return to lab.

II. Collection of Sample

A. To take sample from spigot or tap:

1. Find spigot with direct main connection
2. Put on rubber gloves
3. Flush spigot at full flow for 2 - 3 min. to clear service line
4. If right handed, hold sample bottle near bottom with right hand and remove closure and paper hood with left hand (reverse if left handed). DO NOT LAY CLOSURE DOWN. Hold in such a way to protect closure and bottle from contamination.
5. Allow slip of paper between closure and bottle neck to fall to floor.
6. Thrust bottle into flowing water and allow bottle to fill about 3/4ths full. DO NOT RINSE, especially if bottle contains sodium thiosulfate to neutralize chlorine in sample.

7. Carefully replace closure and hood and secure.
8. Label bottle and place on ice in ice chest for transportation to laboratory.

B. To sample river, stream, lake, etc.

1. Put on rubber gloves.
2. If right handed, hold sample bottle near bottom with right hand and remove closure and paper hood with left hand (reverse if left handed). DO NOT LAY CLOSURE DOWN. Hold in such a way to protect closure and bottle from contamination.
3. Allow paper strip between and bottle to fall to ground.
4. To fill sample bottle
    a. Turn bottle neck opening down and plunge below surface of water quickly to prevent dechlorinating agent from running out.
    b. Turn upward to face bottle opening into current to avoid contamination of water flowing into bottle with samplers hand.
    c. Allow to fill to about 3/4 full. DO NOT OVERFILL especially if bottle contains a dechlorinating agent.
    d. Lift quickly out of water and replace closure and hood.
5. Label bottle and place on ice chest for transportation to laboratory.

II. Common Errors and Affect on Results

A. No dechlorinating agent in bottle. Chlorine activity continues until sample tested so bacteria continue to die and coliform determination gives count which is lower than actual.

B. Sample not chilled when taken. Bacteria continue to multiply, so coliform determination gives count which is higher than actual.

C. Bottle or closure contaminated. Extra bacteria introduced, so coliform determination may give count which is higher than actual.

D. Sample not examined within 6 hrs. of collection. Bacteria will begin to die, so coliform determination will give counts which are lower than actual.

Module No:	**Module Title:** Basic Laboratory Skills
	Submodule Title: Microbiology Skills
Approx. Time: 1/2 hour	**Topic:** Microbiological Dilution Techniques

Objectives:

Upon completion of this module, the participant should be able to:

1. Demonstrate the ability to aseptically prepare a serial dilution of a sample, given all necessary equipment and reference material.
2. Identify precautions which must be taken to prevent contamination at each point of the dilution series.

Instructional Aids:

Handout: Microbiological Dilution Techniques

Laboratory Practice

Instructional Approach:

Lecture
Discussion
Demonstration and laboratory practice

References:

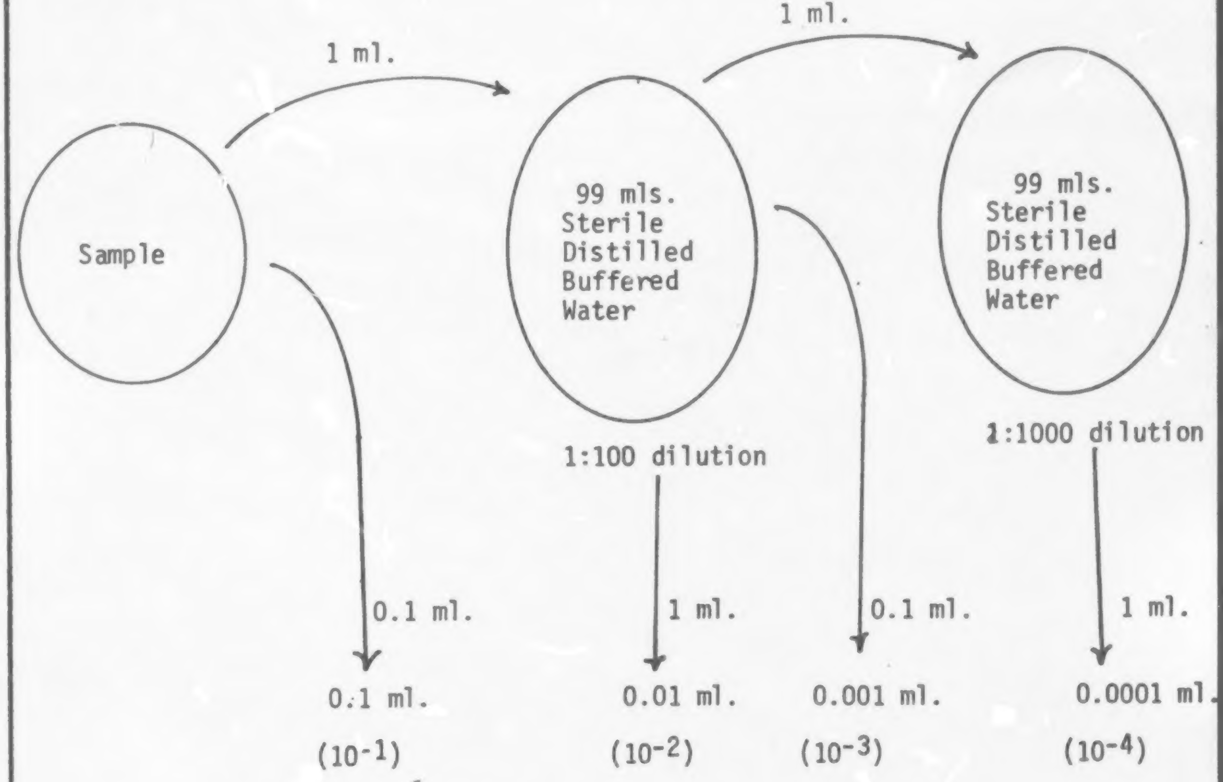
Standard Methods for the Examination of Water and Wastewater

Class Assignments:

Complete laboratory assignment
Read handout

Module No:	Topic: Microbiological Dilution Techniques
Instructor Notes:	**Instructor Outline:**
Handout: Microbiological Dilution Techniques	
1. Emphasize: a. Aseptic technique b. Precision and accuracy	1. Discuss and demonstrate the proper technique for aseptically preparing a serial dilution for microbiological analysis.
	2. Discuss the equipment needed for preparing serial dilutions.
	3. Discuss areas where error is most likely and the effect of errors on results.

MICROBIOLOGICAL SAMPLE DILUTION (Serial Type Dilution)


1 ml.
1 ml.
Sample
99 mls. Sterile Distilled Buffered Water
99 mls. Sterile Distilled Buffered Water
1:100 dilution
1:1000 dilution
0.1 ml.
1 ml.
0.1 ml.
1 ml.
0.1 ml.
(10^{-1})
0.01 ml.
(10^{-2})
0.001 ml.
(10^{-3})
0.0001 ml.
(10^{-4})

A. Use Aseptic Technique throughout Procedure

B. Place 0.1 ml sample into culture tube for 0.1 ml dilution

C. For 0.01 ml sample volume
   1. Place 1 ml sample into a 99 ml dilution blank
   2. Shake vigorously 25 times in an arc of 12"
   3. 1 ml of this 1:100 dilution represents 0.01 ml of original sample

D. For 0.001 ml sample volume deliver 0.1 ml from 1:100 dilution into the culture tube.

E. For 0.0001 ml sample volume

1. Place 1 ml of the 1:100 dilution into a fresh 99 ml dilution blank.
2. Shake vigorously 25 times in an arc of 12"
3. 1 ml of this 1:10,000 dilution represents 0.0001 ml original sample volume.

F. For 0.00001 ml sample volume deliver 0.1 ml from the 1:10,000 dilution into the culture tube.

III. Precautions

A. All volume measurement must be accurate

B. Any measurement error will be compounded in later steps

C. Transfer sample volumes aseptically because any contamination will be carried through entire process.

Module No:	Module Title: Basic Lab Skills
Approx. Time: 1 hour	Submodule Title: General Skills
	EVALUATION - Part A

Objectives:

Upon completion of this module, the participant should be able to correctly answer 75% of the following evaluation questions.

Evaluation Questions

Answer the following questions by choosing the best answer or filling in the blanks.

1. Goggles are worn to

_____a. Protect the eyes

_____b. Protect the hands

_____c. Protect the lungs

_____d. Protect the feet

2. When diluting an acid with water

_____a. Always add the water to the acid

_____b. Mix alternately in a third beaker

_____c. Always add the acid to the water

_____d. Heat on a hot plate

3. Broken glassware

_____a. Can be used if you are careful

_____b. Should be immediately disposed of in the proper waste can.

_____c. Wrapped with electrical tape before using

_____d. Handled with tongs or asbestos gloves

4. Laboratory notebook entries should be:

_____a. Recorded ball point pen

_____b. Recorded on the day the work was done

_____c. In cronological order

_____d. All of the above

5. Why must all containers be properly labeled?

_____a. To identify contents

_____b. To prevent accidents by misuse

_____c. Both a and b

_____d. Containers are not labeled

6. A general format for labeling reagent bottles includes:

a.______________________________

b.______________________________

c.______________________________

d.______________________________

7. A general format for labeling sample bottles includes:

_____a. Sample site, time and date

_____b. Sample site and samplers name only

_____c. Sample type, preservation method, sampler

_____d. Both a and c

8. What does CAP represent in sampling?

_____a. Cleanliness, accuracy, preservation

_____b. Caution - Animal preserve

_____c. Clean appropriate packaging

_____d. Careful and precise

9. Grab samples are

_____a. The same as composite samples

_____b. Taken at a specific time with no regard to flow rate

_____c. Representative of the sewage over a period of time

_____d. Of no value in water or wastewater evaluation

10. What 3 things does sample preservation retard?

a.____________________________

b.____________________________

c.____________________________

11. Identify:

a. An erlynmeyer flask

b. A 2 liter volumetric flask

c. A 500 ml beaker

d. A watch glass

e. A gooch crucible

12. Match

_____a. Water	1. $CaCl_2$
_____b. Sulfuric acid	2. Na
_____c. Calcium chloride	3. C
_____d. Sodium	4. H_2O
_____e. Carbon	5. H_2SO_4

13. As temperature increases, what happens to the volume of a liquid with respect to weight?

_____a. It decreases

_____b. Nothing

_____c. It increases

_____d. It turns to a solid

14. Hydroscopic chemicals

_____a. Pick up water from the atmosphere

_____b. Are always blue in color

_____c. Should be stored in a desicator

_____d. Both a and c are correct

15. What is the concentration in the following solutions:

    a. .1 gram CaCl dissolved in 1 liter of water gives a concentration of __________ mg/l CaCl.

    b. 10 grams of peptone dissolved in 100 grams of water gives a concentration of __________ percent peptone.

16. How much actual sample does 0.1 ml of the 1:100 dilution represent?

    _____a. .1 ml

    _____b. .01 ml

    _____c. .001 ml

    _____d. .0001 ml

17. What is an incubator used for?

    _____a. Drying chemicals

    _____b. Storing reagents

    _____c. Growing bacteria

    _____d. Preserving samples

    _____e. Killing bacteria

18. When installing an incubator, care must be taken to:

    _____a. Install in a vibration free area

    _____b. Install in direct sunlight

    _____c. Keep a pan of dry-rite in the bottom of the incubator

    _____d. All of the above

19. Weigh given object on triple beam balance (with range of 1 - 100 g).

20. Weigh given object on an analytical balance.

Module No:	Topic: EVALUATION - Part A

Instructor Notes:	Instructor Outline:
	Upon completion of the General Skills module the instructor shall give the participant evaluation Part A to complete.
Answers: 1. a 2. c 3. b 4. d 5. c 6. a. Chemical name b. Symbol c. Concentration d. Date prepared e. Prepared by 7. d 8. a 9. b 10. a. Biological action b. Chemical change c. Volatility	
11. a. Erlynmeyer flask b. Volumetric flask c. Beaker d. Watch glass e. Gooch crucible	11. Instructor shall provide a variety of glassware from which the student must choose the correct items.

Module No:

Topic:
EVALUATION - Part A

Instructor Notes:	Instructor Outline:
12. a. 4	
b. 5	
c. 1	
d. 2	
e. 3	
13. c	
14. d	
15. a. 100	
b. 10	
16. c	
17. c	
18. a	
19. Result shall be ± 0.1 gram	19 & 20. Instructor shall provide weights
20. Result shall be ± 0.002 gram	

Module No:	Module Title: Basic Lab Skills
	Submodule Title: Chemistry Skills
Approx. Time: 1 hour	EVALUATION - Part B

Objectives:

Upon completion of this module the participant should be able to correctly answer 75% of the following evaluation questions.

Evaluation Questions

Choose the best answer

1. Accuracy is a measure of how close your answer is to the true answer.

_____a. True

_____b. False

2. Most forms of volumetric analysis include some form of color measurement.

_____a. True

_____b. False

3. Precision and accuracy mean the same thing.

_____a. True

_____b. False

4. All forms of volumetric analysis include a titration.

_____a. True

_____b. False

5. Rate in order of increasing accuracy.

_____a. 250 ml erlynmeyer

_____b. 250 ml volumetric flask

_____c. 250 ml graduated cylinder

6. Rate in order of increasing accuracy.

_____a. 10 ml mohr pipet

_____b. 10 ml volumetric pipet

_____c. 10 ml beaker

_____d. 10 ml graduated cylinder

7. Volumetric flasks are calibrated to contain

_____a. True

_____b. False

8. A 100 ml volumetric pipet and a 100 ml volumetric flask have the same accuracy and may be used interchangeable.

_____a. True

_____b. False

9. A graduated cylinder may be calibrated to deliver or to contain.

_____a. True

_____b. False

10. Given the normality and volume of a solution and the volume of a second neutralizing solution may be calculated.

_____a. True

_____b. False

11. Given the equivalent weight of a dissolved chemical and the volume that it is dissolved in, the normality of the solution can be calculated.

_____a. True

_____b. False

12. Adsorbance is inversely proportional transmittance.

_____a. True

_____b. False

13. The concentration of a colored solution is directly proportional to:

_____a. Its transmittance

_____b. Its absorbance

_____c. Both a and b

_____d. Neither a nor b

14. Adsorbance or transmittance of a sample is not affected by:

_____a. Turbidity

_____b. Diameter of sample tube

_____c. Amount of sample in tube

_____d. Type of sample tube

15. A standard curve is used to convert adsorbance or transmittance readings to concentration.

_____a. True

_____b. False

16. A standard curve may be made on any type of graph paper.

_____a. True

_____b. False

17. To obtain a straight line plot in colorametric analysis from a series of transmittance/concentration values __________ graph paper must be used.

_____a. Log-log

_____b. Semi-log

_____c. Linear

18. Indicate which of the following are EPA approved standard references.

_____a. Standard Methods, 14th Edition

_____b. Methods for chemical analysis of wastewater, EPA.

_____c. Simplified Methods for Wastewater Analysis , WPCF _____d. Simplified Methods for Water Analysis, AWWA.

_____e. ASTM Methods, Part 31

19. Changes in Standard Methods are official only when published in the federal register.

_____a. True

_____b. False

20. Order the following list of equipment using the model order form and the laboratory supply catalog provided by the instructor.

a. 400 sterile, disposable, glass, single wrapped, 10 ml pipets

b. 3-1000 ml class A, glass stoppered volumetric flasks

c. 24 milk dilution blanks with screw caps and 99 ml markings

d. 12-250 ml griffin beakers - heavy duty

e. 5 large tip mohr pipets

Supplier:

Quantity	Catalog Number	Description	Unit Price	Total Price

Module No:

Topic: EVALUATION - Part B

Instructor Notes:

Instructor Outline:

Answers:

1. a
2. b
3. b
4. a
5. a. 1
   b. 3
   c. 2
6. a. 3
   b. 4
   c. 1
   d. 2
7. a
8. b
9. a
10. a
11. b
12. b
13. b
14. c
15. a
16. a
17. b
18. a, b, & e
19. a

Module No:	Topic: EVALUATION - Part B

Instructor Notes:	Instructor Outline:
20. The form shall be completed correctly with respect to: a. Quantity b. Catalog number c. Description d. Price e. Supplier name and address	20. The instructor shall provide the laboratory supply catalog of his choice providing all glassware listed is available from that supplier. The instructor shall also develop answer key from that catalog.

Page 1 of 4

Module No:	Module Title: Basic Lab Skills
	Submodule Title: Microbiology Skills
Approx. Time: ½ hour	EVALUATION - Part C

Objectives:

Upon completion of this module, the participant should be able to correctly answer 75% of the following evaluation questions:

Evaluation Questions

Answer the following questions by choosing the best answer.

1. What is the major purpose of a disinfectant?

_____a. To pick up dust with

_____b. To lower the number of viable organisms on a surface.

_____c. To wash glassware in

_____d. To

2. Kraft paper is used for packaging equipment for hot oven sterilization.

_____a. True

_____b. False

3. Only distilled water is used for preparation of microbiological growth media.

_____a. True

_____b. False

4. The balance used to weigh microbiological media and reagents must:

_____a. Have a 0.5 gram accuracy at a 150 gram load.

_____b. Have a 1 gram accuracy at a 200 gram load

_____c. Be an analytical balance

5. An autoclave has the capability of exploding while operating.

_____a. True

_____b. False

6. An autoclave may be loaded to a maximum of:

_____a. 100% capacity

_____b. 40% capacity

_____c. 80% capacity

_____d. 30% capacity

7. Liquids are always sterilized in a

_____a. Sterilizing oven

_____b. Steam sterilizer (autoclave)

8. Normal sterilization cycle in a sterilizing oven is

_____a. 15 min. at 170° C.

_____b. 1 hour at 121° C.

_____c. 15 min. at 121° C.

_____d. 1 hour at 170° C.

9. To remove dust from a microscope lens do not use:

_____a. A lens tissue

_____b. A camel's hair brush

_____c. A clean handkerchief

_____d. A quick blow of clean air

10. Microscopes may be carried one in each hand.

_____a. True

_____b. False

11. Unsterile pipets may be used in making aseptic transfers.

_____a. True

_____b. False

12. Why is a sample tap flamed with a propane torch?

    _____a. To incinerate the bacteria

    _____b. To burn off chemical contaminants

    _____c. Sample taps are not flamed

    _____d. To melt plastic seals

13. What is the dechlorinating agent used in samples collected for microbiological testing?

    _____a. **Sodium hydroxide**

    _____b. Potassium phosphate

    _____c. Sodium thiosulfate

14. What is the type of dilution used in microbiological sample dilution.

    _____a. Parallel

    _____b. Serial

15. Identify the following parts of a microscope on the microscope provided by the instructor.

    a. Eye piece

    b. Oil immersion lens

    c. Course adjustment

    d. Stage

    e. Condenser

    f. Light source

16. Make an aseptic transfer using the equipment provided by the instructor.

Module No:	EVALUATION - Part B

Instructor Notes:	Instructor Outline:
	Upon completion of the Microbiological Skills module the instructor shall give the participant Evaluation Part C to complete.
Answers: 1. b 2. b 3. a 4. a 5. a 6. c 7. b 8. d 9. c 10. b 11. b 12. a 13. c 14. b	
15. a. eye piece b. oil immersion lens c. course adjustment d. stage e. condenser f. light source	15. The instructor shall provide a microscope from which the students shall identify the parts given.
16. Performance acceptable to instructor	16. The instructor shall provide all the necessary equipment for proper aseptic transfer of a sample.